The Effects of Delays and Interference on Spatial Working Memory in Differentially Reared Rats.

A THESIS SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ARTS IN PSYCHOLOGY

by

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1994

Acknowledgements.

I wish to thank John Dalrymple-Alford, my supervisor, for his guidance, patience and perseverance without which this research would not have been possible. I also wish to thank Patricia Meatchem whose professional advice was invaluable during this research, and Mathew Byers for his general assistance and support.

Contents

Abstract.

This Thesis investigated the effects of three forms of differential housing, namely Isolated (IC), Enriched (EC), and Long-term Enriched (LTE), on short-term spatial working memory, by using a series of delays and two forms of explicit interference to test retention of the basic non-matching-to-sample task. Experiment 1 investigated the behaviour of two groups of rats, IC and EC, which had been differentially housed from 35 days of age and found that the longer the delay, the more performance was impaired across both housing groups. The Maze interference was found to significantly affect performance, but the Interference Box did not, indicating that the nature of the interfering task was of prime importance. No effects for differential housing emerged. Experiment 2 investigated the behaviour of three experimental groups, IC, EC and LTE, which had been differentially housed from 21 days, and, likewise, found that the delays significantly affected performance, as did both forms of Interference. Analysis of the behaviour within the Box Interference found that Experiment 2 animals exhibited more activity, giving one possible explanation for this difference in behaviour between the two experiments. An open field activity observation was also done. The results of these experiments did not support the notion that an enriching environment produces superior cognitive capacity.

1. INTRODUCTION

Housing animals in different kinds of stimulating environments has been found to have profound effects upon anatomy, behaviour, learning and memory. This Thesis investigated the effects of differential rearing on learning and memory in rats by using a simple T-maze working memory task, and employed time delays and interference to test retention performance in animals reared under different conditions.

A wide variety of stimulus enrichment and deprivation conditions have been utilised in previous research which, on a continuum, can vary from sensory deprivation through to evidently complex conditions. As such, the nature of the stimulus/deprivation is evaluated in terms relative to the standard laboratory environment (Renner & Rosenzweig, 1987; Rosenzweig, Bennett & Diamond, 1972). There are general guidelines to which most research facilities adhere, and these ensure a minimum of discomfort to the subjects while maintaining good health. However, due to methodological and resource constraints these housing conditions do not always resemble those found in the natural environment, (Bradshaw & Poling, 1991).

Different Environmental Conditions

The isolated environment or condition (IC) is generally self explanatory, with the subjects being maintained singly with no physical contact with other members of the species. Stimulation of any form is usually kept to a minimum, with limited opportunity for physical exertion due to the cage dimensions, and only routine maintenance to limit extracage excitation. Cage construction and size can vary somewhat between studies (For examples see: Bennett, Rosenzweig & Diamond, 1970; Menich & Baron, 1984; Morgensen, 1991; Rosenzweig & Bennett, 1976).

The social environment (SC) is the housing condition which most resembles the standard colony situation in many research facilities and consists of a standard laboratory cage containing, generally two to six animals of the same gender. Although the cage size and number of animals may vary, the subjects, much like in the isolated condition, are given little or no extracage stimulation (For examples see: Menich & Baron, 1984; Renner & Rosenzweig, 1987; Rosenzweig and Bennett, 1976).

Of all the housing conditions, the enriched environment or condition (EC) has the most diversity within the literature. Usually there are five to twelve animals per cage which could vary from a large cage (Einon, 1980) to a large room (Sharp, Barnes & McNaughton, 1987). One experimenter used interlocking chambers of differing sizes with connecting tunnel systems (Morgensen, 1991). The one variable which links all these studies is the use of objects placed within the enclosures for the animals to "play with" and manipulate. This assortment of objects is changed regularly, often daily, from a pool of suitable objects. (eg. Juraska, Handerson & Muller, 1984; Pacteau, Einon & Sinden, 1989; Renner & Rosenzweig, 1987, Rosenzweig & Bennet, 1976).

Research using these differential housing conditions has shown that reliable differences in some behavioural measures and anatomical structures can be gained in animals which were previously genetically identical but were reared or placed in different environmental conditions. In such cases, any biological differences are implied to be due to this environmental influence. The following section provides a brief overview of the anatomical and behavioural changes which can be seen in response to differential experience.

ANATOMICAL CHANGES

Many physical aspects of an organism can be changed by experience. One reliable finding is that rats maintained in an isolated environment are heavier than those from an enriched habitat

(Einon & Morgan, 1978b; Menich & Baron, 1984; Renner & Rosenzweig, 1987). The internal organs (e.g. heart, liver, spleen, and testicles) have also been seen to develop more in impoverished rats. This increase in size naturally reflects a likewise increase in the skeleton, including the external dimensions of the skull, but not the intracranial dimensions (Renner & Rosenzweig, 1987). Menich & Baron (1984) found that their socially housed animals also lived longer than the isolated subjects.

Of all the central nervous system anatomical changes, the increase in gross cortical weight is the most obvious. Enriched animals may display an increase in cortical weight of up to 5% (Bennett et al, 1970; Renner & Rosenzweig, 1987; Walsh & Cummins, 1975; Widman & Rosellini, 1990). This change is by no means equal across all brain regions. The area of largest magnitude of environmental effect is the occipital region of the cortex where differences can reach a mean of eight or nine percent between enriched and isolated animals (Bennett, Diamond, Kreach & Rosenzweig, 1984; Renner & Rosenzweig, 1987; Rosenzweig & Bennett, 1976; Walsh & Cummins, 1975; Widman & Rosellini, 1990). Unlike the effects on the cortex, environmentally induced alterations in other brain regions may vary. Changes at the level of the sub cortex can be rather small, and have been opposite in direction from the usual effects in the cortex; enriched rats have produced a slightly lesser weight of sub cortex although they generally have a greater weight of cortex (Bennett et al, 1970).

The density, or number, of neurones in EC animals have been found to be lower, per unit of tissue, than the levels found in IC rats (Bennett et al, 1970; Renner & Rosenzweig, 1987; Rosenzweig et al, 1972), so cellular multiplication is not responsible for the variation in tissue weight between EC and IC subjects. This absence of disparity in neurone number therefore suggests some differences in the characteristics of the individual cells (Renner & Rosenzweig, 1987).

Cross-sections of individual neurons have shown cell bodies and nuclei in EC rats to be up to 13% larger (Bennett et al; 1970; Renner &

Rosenzweig, 1987; Rosenzweig et al, 1972; Walsh & Cummins, 1975), indicating higher metabolic activity (Renner & Rosenzweig, 1987; Rosenzweig et al, 1972). This implication is given support by changes evident in glial cells which provide metabolic support for neuronal activity. Glial cells have been found to increase in number in EC subjects; oligodendroctye type cells account for most of this environmentally induced difference rather than the astrocytes, although it is evident that these too increase in number, but to a smaller magnitude (Renner & Rosenzweig, 1987; Rosenzweig & Lieman, 1982).

Rats exposed to enriching environments have shown evidence of increased dendritic branching (Juraska et al, 1984; Murtha et al, 1990; Rosenzweig, 1984; Walsh & Cummins, 1975). This arborization of the dendrites has been used to explain some of the alterations in cortical volume between EC and IC rats, but other evidence indicates that this increased dendritic branching in enriched animals may occur within an equal area as that employed by the dendrites of isolated subjects (Greenough & Volkmar, 1973; Renner & Rosenzweig, 1987). The EC-IC variation in dendritic arborisation becomes more distinct as the order of dendritic branching increases: an order-one branch stems directly from the cell body, the first bifurcation of that dendrite indicates second-order dendrites etc. Using this categorisation method researchers found that enriched animals display consistently more higher-order dendritic branches in the pyramidal neurons (layers II, IV and V) and stellate neurons (layer IV) than their IC counterparts (Greenough & Volkmar, 1973; Renner & Rosenzweig, 1987; Volkmar & Greenough, 1972).

Because dendrite branches are locations for synaptic junctions, a change in the number of dendrites in EC rats would imply a corresponding increase in the number of synapses in these animals, which has been found in some studies (i.e. Sirevaag & Greenough, 1986; Walsh & Cummins, 1975), but not in others (Diamond, Linder, Johnson, Bennett & Rosenzweig, 1975), (cited in Renner & Rosenzweig, 1987). The type of neurone, the shape of the synapse (i.e. regular or irregular) and shape of the vesicles have been found to be significant in determining environmental effect at the synaptic

cleft. The criteria for the selection of the synapse to be measured has had great variability over studies, and this may contribute to the lack of agreement between researchers, but these considerations will not be delt with here (for a review see Renner & Rosenzweig,1987).

The cholinergic system is known to be involved in many behavioural processes, including learning and memory (Beninger, Jhamandas, Boegman & El-Defrawy, 1986). Cortical levels of cholinesterase have found to be higher in animals trained in learning tasks, and in enriched animals. Acetyl cholinesterase (AChE) activity due to differential rearing is not as clear cut, although it appears that the cortical/subcortical ratios are relatively stable with slightly lower levels of AChE in the cortex, and slightly higher levels in the sub cortex in EC animals (Bennett et al, 1970; Bennett, Diamond, Krech & Rosenzweig, 1974; Renner & Rosenzweig, 1987; Rosenzweig, 1984; Widman & Rosellini, 1990).

BEHAVIOURAL FINDINGS

The anatomical changes found in animals exposed to differential environments seem likely to indicate underlying behavioural differences. Rats are highly social animals, and as a result the quality and quantity of social interaction must be affected by social isolation and by degrees of enrichment (Einon, Morgan & Kibbler, 1978). Einon, Humphreys, Chivers, Field and Naylor (1981) queried whether the long term effects of social isolation were due to deprivation of the opportunity to engage in social play and found that their results were consistent with the implication that more severe effects of social isolation were found in species that engage in social play.

Day, Seay, Hale and Hendricks (1982) looked into the unrestricted behaviour of isolated and social rats when placed together in an observation cage and found significant differences in aggressive types of behaviour with the IC animals displaying significantly higher mean frequencies of domination, boxing and fighting. Impoverished subjects also elicit more aggressive behaviours than

group reared animals, even when introduced into stable social groups (Day et al, 1982).

Isolated animals also tend to have a generally higher level of activity in both non-specific activity over a 24 hour period (Dalrymple-Alford & Benton, 1981) and in an open field (Dalrymple-Alford & Benton, 1984a; Dell & Rose, 1987; Einon & Morgan, 1976). Although some studies have found isolates to be less active (Einon et al, 1981; Menich & Baron, 1984), Dalrymple-Alford & Benton (1981) suggest that this may be due to higher levels of fear responses such as freezing at the beginning of open field testing and that longer periods in the field and repeated testing over days more readily exhibit the characteristic hyperactivity of the isolated animals.

When given access to an enriching environment, subjects who have been raised in a social condition tend to have more complex interactions with the objects than do isolation reared rats (Renner & Rosenzweig, 1987). Social rats tend to contact more objects, a greater variety of objects as well as climb, move and paw more objects. They aslo tend to have a higher level of manipulatory kinds of contacts with objects than do isolates (Dalrymple-Alford & Benton, 984b; Einon & Morgan, 1976; Einon, Morgan & Kibbler, 1978; Widman & Rosellini, 1990). Myhrer, Utsikt, Fjelland, Iversen & Fonnum (1992), however, found that overall IC animals spent more time in total exploring objects, and their general surroundings, than did socially or enriched reared rats, although the socially reared subjects preferred novel objects to neutral objects significantly more than either IC or EC animals.

In visual discrimination tasks the performance of isolated animals matches that of the enriched (Bennet et al, 1970; Mogensen, 1991), but when the discrimination problem is reversed then EC animals generally make considerably fewer errors per reversal problem and solve significantly more reversal problems (Bennett et al, 1970). The same pattern of results has also been found with motor transfer tests; enriched animals are generally superior when required to remove an obstruction in a different way from that which had been learned (Einon, Morgan & Kibbler, 1978; Renner &

Rosenzweig, 1987). This performance difference can be reduced if the animals are trained in a number of distinct phases or given successive exposure to the same transfer problem (Einon, Morgan & Will. 1980).

The Hebb-Williams maze usually consists of a square field marked off into smaller squares, with different barrier configurations and a start and goal box at diagonal corners. A series of maze problems are presented to the rats and the performance is seen to reflect the level of problem solving ability of each animal (Renner & Rosenzweig, 1987). The results with this maze indicate a consistent superiority of EC raised animals over their IC counterparts, as IC rats make significantly more errors even over repeated trials (Cummins, Walsh, Budtz-Olsen, Konstantinos & Horsfall, 1973; Dalrymple-Alford & Benton, 1984b; Dell & Rose, 1986; Murtha, Pappas & Raman, 1990).

The radial maze which is used to test aspects of spatial memory has also exhibited similar results with the EC subjects performing more accurately by making significantly less errors than their IC counterparts (Einon, 1980; Einon et al, 1980; Juraska et al, 1984; Pacteau et al, 1989). Extended training, however, will lessen these group differences (Bolhuis, Bijlsma & Ansmink, 1986).

Age and Duration of Exposure:

A perusal of the literature shows a tendency for researchers to begin their environmental studies when the animals are weaned which is usually between the ages of 21 to 25 days (for examples see Dell & Rose, 1986; Dalrymple-Alford & Benton, 1984a; Bennett et al, 1974), although some have started their animals as early as 16-17 days (Einon & Morgan, 1976; Einon & Morgan, 1978b), and some as late as 32 months (Sharp et al, 1987). The duration of exposure to the rearing environments also shows some variation throughout the literature with some varying from 20 days (Einon et al, 1978) to 40 days (Schenk, Hunt, Colle & Amit, 1983), with the longest being 160 days (Bennett et al, 1970).

Naturally, the age of exposure to differential environments will affect both the anatomical and behavioural results, with the greatest degree of effect being generated when the animals are exposed from 21 days of age for a period of 30 days, or until the age of 50 - 51 days. This is often regarded as a sensitive period for maximal environmental results, and even a reversal of the environmental treatment does little to alleviate or enhance performance on learning tasks (Bennett et al, 1970; Dalrymple-Alford & Benton, 1981; Dalrymple-Alford & Benton, 1984b; Day et al; 1982; Einon et al, 1981). Likewise, if exposure to the differential environments occurs after the age of 60 days little difference in scores on learning tasks is generally found (Bennett et al, 1970).

The characteristic hyperactivity of isolation raised animals also remains intact despite subsequent group housing, and socially reared animals do not generally develop hyperactivity when later put into an impoverished environment (Dalrymple-Alford & Benton, 1984b; Einon, 1980; Einon & Morgan, 1978b). The same trend appears in performance on the Hebb-Williams maze (Dalrymple-Alford & Benton, 1984b), and on the radial maze (Einon, 1980) with the social raised animals still making fewer errors than isolated raised animals, despite the social animals being isolated at the time of testing.

Other behaviours of both rehoused groups can change to some extent however, with emergence latencies being affected by the type of housing at the time of testing (Dalrymple-Alford & Benton, 1984b). By contrast, animals who have been exposed to a partial isolation environment where they are allowed one hour of social contact per day tend to have more intermediate scores on measures of hyperactivity and object contact, even after they have been placed in total isolation for seven weeks (Einon et al, 1978). In summary, some of the behavioural consequences of social isolation in rats are only found if isolation is carried out during a post-weaning sensitive period and these effects are not reversed by subsequent social housing (Bernstein, 1979; Dalrymple-Alford & Benton, 1984b; Day et al, 1982; Einon, 1980; Einon & Morgan, 1978b, Einon et al, 1978).

Interpretation of the Behavioural Differences:

The behavioural differences between enriched and impoverished animals on behavioural and memory tasks suggest some superiority of the EC subjects memory abilities over that of the IC subjects. Renner & Rosenzweig (1987) have suggested that the greater the task complexity the greater the likelihood that behavioural differences between EC and IC raised animals will be found, and that relatively simple tasks do not yield consistent EC-IC differences. For example, in conditioned taste aversion no significant differences were found between enriched and isolated animals in acquisition of this task (Giardini, 1985; Renner & Rosenzweig, 1987). Whereas, in the Hebb-Williams maze which was designed to test the problem solving skills of the rat, the performance of enriched animals is consistently superior to that of their isolated counterparts (Dell & Rose, 1986; Juraska, Henderson & Muller, 1984; Renner & Rosenzweig, 1987).

These same patterns of EC superiority/IC inferiority emerge in performance on tests of spatial memory, including the Lashly III maze (Bennett et al, 1970; Renner & Rosenzweig, 1987; Widman & Rosellini, 1990), the Morris water maze (Saari et al, 1990), and the Radial maze (Einon et al, 1980; Juraska et al, 1984; Pecteau et al, 1989). These differences in competency on these maze tasks have also been interpreted as suggesting an enhancement of spatial aptitudes, or spatial memorial capacity in enriched animals (Einon, 1980; Pacteau et al, 1989).

Dell & Rose (1986), however, state that EC/IC differences in maze performance do not necessarily indicate evidence for differing cognitive capacity or problem solving ability. Performance on tasks such as the Hebb-Williams maze and the Radial maze are not a pure indication of memory ability, but also include an indication of the animals ability to alter their response strategy in answer to the changing nature of the task. There are also experimental difficulties in seperating out variations in learning, or cognitive capacity per se from performance related variables such as motivation, arousal and activity levels, as well as differences in motor and sensory

capacities. For example, some of the behavioural differences found between EC and IC subjects can be explained by a lack of response inhibition in IC subjects, and this combined with the characteristic hyperactivity associated with isolation is evident in innapropriate exploration in maze tasks which in many cases leads to an increase in errors (Dalrymple-Alford & Benton, 1984b; Einon & Morgan, 1978a; Einon & Morgan, 1978b; Rose, Love & Dell, 1986).

The differences in behaviour between the differentially raised animals may also suggest differences in declarative and procedural memory systems. Procedural memory is implicit, is only available through performance, and involves knowledge about how to perform various cognitive and non-cognitive activities. Some examples of this form of memory system include skill learning and simple classical conditioning. Whereas declarative memory, at least in humans, is available to conscious awareness and includes knowledge about the facts and specific episodes learned in everyday experience (Anderson, 1980; Schacter, 1987; Shimamura, Salmon, Squire & Butters, 1987; Squire & Zola-Morgan, 1985).

Within the animal literature declaritive memory is further divided into working memory and reference memory. Working memory involves the recall of recent events, and as such can only be exhibited in animals through performance in tasks which use procedures which involve exposure to events of transient importance. Tasks using trials in which information is useful for only that trial taps working memory and includes such tasks as T-maze alternation. Reference memory, however, refers to information stored over the long term, and spatial discrimination tasks which utilse information which is useful over all trials are used to test for this aspect of memory (Beninger et al, 1986; Squire & Zola-Morgan, 1985; Tulving, 1987).

Most of the memory tasks mentioned previously include both the procedural and declarative aspects of memory, and as such it can be difficult in separating out those aspects of memory which may be specifically affected by exposing animals to different rearing environments. Any differences between experimental groups in their ability to acquire a memory task may indicate differences in

procedural memory. Performance differences in the working memory aspects of declarative memory are highlighted in procedures which investigate rates of forgetting within the context of working memory tasks. Forgetting is an important aspect of testing memory abilities, especially in working memory tasks in which delays or interference are employed.

The imposition of a time delay within the context of a working memory task will highlight differences in rates of forgetting between experimental groups. Delay periods used within the literature have varied considerably from 2 seconds (Dunnett, 1990; Dunnett, Evenden & Iversen, 1988), to 24 hours (Bolhuis, Bijlsma & Ansmink, 1986). Researchers have found that the longer the imposed delay, the worse the performance on working memory tasks, whereas, delays appear to have no effect on reference memory (Beatty & Shavalia, 1980; Beninger et al, 1986; Hepler, Olton, Wenk & Coyle, 1985; Tran & Beatty, 1985).

Rates of forgetting on a working memory task may also be tested by the imposition of a form of interference within the task. The effect of differing forms of interference on both working and reference memory has also been tested with mixed results. Whether the interference is proactive (Gordon, Bremman & Schlesinger, 1976) or retroactive (Cook & Brown, 1985), related (Tran & Beatty, 1985) or unrelated to the original memory task (Jarrard, 1975), how much information the rat has to remember from the original task, the duration of the interference (Cook & Brown, 1985), and how long the delay is in which the interference is placed (Beatty & Shavalia, 1980) have all combined into an immensely complex picture. How interference would effect performance on a working memory task in differentially reared animals has yet to be systematically examined.

The Present Study

The purpose of this thesis was to investigate the working memory of rats which had been exposed to differential rearing environments. The first experiment tested animals which had been raised under two differing environmental conditions (EC and IC) from

the age of 35 days, for 30 days. A nonmatching-to-sample T-maze task was utilised in effort to produce a simple test of working memory, which when incorporated with various delays and interferers, would emphasise the rate of forgetting among the subjects and not the rate of learning as would a more complex task. It would be expected that with the incorporation of the delay and interference the more likely that differences between rats raised in an enriched environment and those reared in isolation would be evident, without affecting performance on the basic memory task itself.

Because these first experimental animals were housed well into the period regarded most sensitive for the development of differential behaviour, and then isolated for the duration of the behavioural testing a second experiment was considered appropriate. The second experiment looked at behavioural differences between rats raised in different environments from 21 days of age, for 30 days. A third experimental group was also added, namely a Long-term Enriched Condition (LTE) which were allowed access to an enriching environment throughout the entire experiment, to control for the effects of isolation at time of testing. Differences in open field behaviour and gross brain measurements were investigated.

2. EXPERIMENT 1

Method

Subjects:

Twenty Female Albino (Sprague-Dawley) rats were bred and raised in the Animal Facility in the Department of Psychology. They remained undisturbed with their mother and siblings until the age of 21 days at which time they were segregated into standard colony groups of 4 single sex animals per cage. At the age of 35 days matched animals were assigned to the isolation or enrichment condition based on litter, age, and weight, such that each subject had a corresponding litter-mate in the other environment.

Housing Conditions:

Ten animals were assigned to the isolated environment (IC) which consisted of a single rat in a standard opaque plastic colony cage (27x43x15cm). Subjects could hear and smell other animals, but not see them and remained undisturbed except for routine maintenance.

The remaining 10 subjects were placed together in a single enriched environment consisting of a large (40.5x100x46cm) metal cage with mesh floor, front and lid, which contained a large assortment (10-15) of objects made of wood, plastic and metal. The varying number of objects were changed and cleaned on a daily basis.

All animals spent 30 days in their respective environments before they were all weighed and then rehoused in individual cages. A neutral staff member then divided the animals into two groups (E & I), each of which had an equal number of enriched and isolated animals organised in random order such that the testing would be done on a blind basis by the researcher to aviod experimenter bias.

All animals were kept in the colony room with a reversed 12 hour light cycle (lights on 1800, off 0600) and had free access to food and water prior to the maze training phase after which the animals were kept at 80-85% of free feeding weight of animals of the same strain, age and sex.

Apparatus:

The two identical T-maze apparatuses were constructed of wood and painted mat grey (for dimensions see Figure 1.), with a 2.3cm metal lip around the entire periphery of the maze. The food wells were set 1cm above the floor of the goal area and were comprised of a 100mm x 50mm block of grey painted wood with a 25mm diameter, 1cm deep depression. The start area was divided from the rest of the maze by a door shield which was designed to prevent the rats from climbing around or over the door and starting prematurely. Choice decision lines were marked on the floor of the apparatus 25cm from each goal area and 10cm from the start area door. Both mazes were raised 86cm from the floor. The T-mazes were both kept in the same experimental room, situated parallel with only 1 metre separating them.

The wooden "interference box" measured 32 x 32cm square x 41cm high, with its interior painted mat grey. Sack cloth was attached to three of the inside walls, and the grey floor was covered with chicken wire. The remaining wall contained an unpainted closed wood door 15cm wide which protruded 1cm into the box. The objects placed inside this "interference box" included a set of stimulus-rich wood goal boxes (designed for an object- recognition experiment), 9 x 12 x 19.5cm, open at both ends and painted in an assortment of colours in differing patterns. These goal boxes contained a variety of objects and materials attached to the inside walls which projected into the goal boxes to provide a wide variety of textures, colours and smells.

Procedure

Pretraining:

The animals were given 3 days adaptation to their individual cages before food deprivation was initiated (day 0). The cages were divided into four squads with five animals per squad; three animals were be run on one apparatus labelled 'A', and two on the other apparatus labelled 'B'. In the next squad the opposite would be the case with two on 'A', and three on 'B'. The rats were always run on their delegated maze.

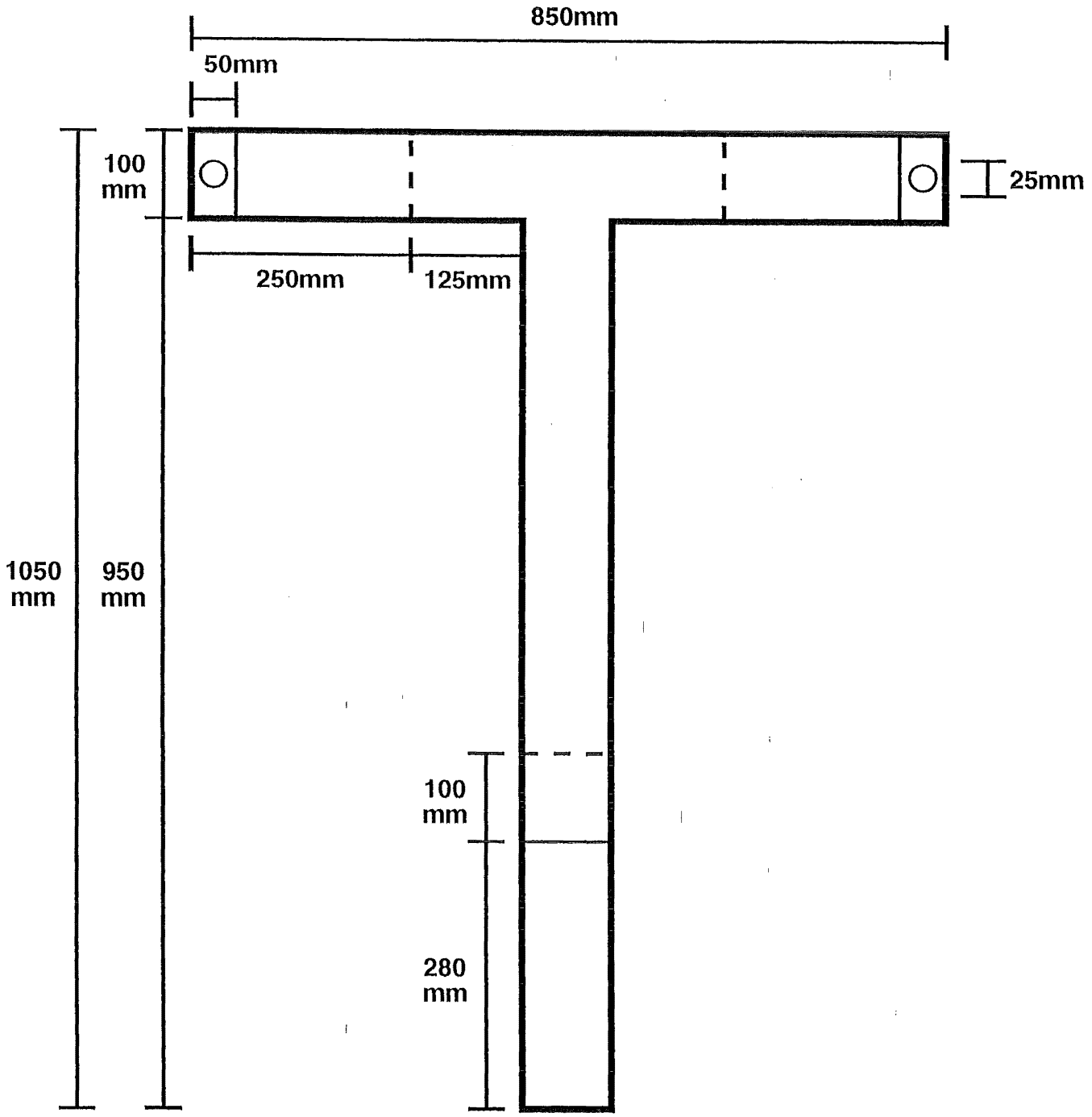

Figure 1 Dimensions of the T-maze apparatus used.

Handling and pretraining commenced the following day with the rats being given 6grams of Nestle chocolate chips (0.1gm each) in their cages to familiarise the animals to the food which would be used for reinforcement. The rats were also handled individually by the researcher for a short period of time (1-2 minutes). For the next three days, the rats were introduced singly for two to three minutes daily to the maze which had chocolate chips scattered along the stem and arms. Again, the rats were handled for a short period on each day.

On the fifth day, the guillotine door was introduced. The animals were placed on the apparatus singly with the door closed, which was then opened to allow the rat to explore the maze. Over the next eight days, this procedure was repeated except the amount of chocolate scattered around the maze was steadily reduced until it remained only in the food wells.

The final two days of pretraining involved the introduction of double run trials down the stem of the maze to the goal areas, using a non-matching-to-sample choice procedure. The left/right positioning for this discrimination task was taken from the sequences given by Fellows (1967) ensuring a balanced but psudo-random positioning within each day. On the information run one of the arms was blocked off with a block of wood allowing access only to the opposite arm. The rat was placed on the maze in the start area, the door was opened and the animal allowed to run down the stem into the unblocked arm to the goal area where the well contained a single piece of chocolate. The guillotine door was closed as soon as the animal has passed through. For the choice run the animal was then placed back in the start area, the block was removed, the door opened and the animal again allowed to run down the stem and choose an arm.

If the rat returned to the same arm it had just previously visited it recieved no reinforcement and was removed from the apparatus back to its home cage. If the animal chose the arm not previously visited the rat received two pieces of chocolate in the food well. The period between the first information run and the choice run was less than 3 seconds but counted as 0 delay.

The rats were run on a rotational basis, first member through to the fifth within each squad until four runs or trials for each rat was complete. The inter trial period was the amount of time required to

run all 5 animals in the squad which was usually 3 minutes. If an animal spent in excess of 2 minutes to make its way to the goal area it was placed back in the start area and allowed to either rerun or spend a further 2 minutes on the maze.

The animals were then exposed to three sets of behavioural tests. The first was the initial T-maze training in the basic task, the second involved the presentation of delays, and finally the imposition of explicit interference into the basic working memory task.

Phase 1: Initial T-maze Training

Initial acquisition of the basic task was established by training each rat for eight double-run trials per daily session, for 12 days.

Phase 2: Delays

Inter-run Delays of 30sec, 60sec, 90sec, and 120sec were then imposed between presentation of the stimulus arm (information run) and the choice run for each trial. Only one delay duration was given for all rats on any one day. The inter-run delays were presented twice each in an ascending followed by a descending order, with each alternate daily session being run with no imposed inter-run delay. This phase consisted of a total of 15 sessions.

Phase 3: Interference

The next phase of this experiment involved the introduction of two types of explicit interference, exposure to a "Maze Interference" or "Interference Box", between the information and choice components of each trial, in conjunction with a 30sec delay. This phase also included an expansion of the run sequence from eight double-run trials per daily session to twelve trials per daily session with two days of each interference condition.

The first interference involved running the rats on the alternate (ie. unfamiliar) T-maze apparatus in the 30 sec inter-run delay. To prepare the rats for running on the unfamiliar maze they were given two days training in which every alternate double-run trial from the sequence of eight was run on the other maze, as per normal with no delay. The rats were then given three days of running on their own maze with a 30sec delay between presentation and choice

components to familiarise the animals to this waiting period without it affecting their performance. This ensured that any effect upon their performance would be due to the interferer and not the time delay. It was at this point where the run sequence was extended from eight to twelve trials per session.

In the "maze interference" sessions the rat ran the forced arm, as per normal, on their "own" maze (either 'A' or 'B'), then they were placed on the second T-maze and forced to run the unfamiliar maze in the opposite direction as their information arm on their regular maze. They were then placed back on their own maze to complete the choice component of the trial with the correct arm being the same as if they had not had the intervening forced run on the second maze. If the animals had merely learned to alternate, then their choice response would be incorrect.

The second explicit interference imposed in the 30sec inter-run delay was the "interference box", which was essentially a mini-enriched environment, containing a series of stimulus-rich goal boxes and a variety of small objects. The rats were placed singly in the "interference box" and allowed 30sec to explore, during which the rats were observed and the type (i.e., manipulatory Vs nonmanipulatory) and number of object interactions were recorded. The manipulatory behaviours recorded were pawing, biting, moving and climbing onto objects; non-manipulatory behaviours included rearing, entering and sniffing objects. Each double-run trial used a different goal-box and objects which differed in their positioning and orientation within the mini-environment. Hence, on each daily session with the "interference box" interferer, the animals were exposed to 12 different sets of stimuli, a total of 24 sets over the two interference sessions.

The interference conditions were presented in the same manner as that of the delays. The accending and descending order of presentation was replicated; the first maze interference session was followed by two box interference sessions, followed by the second maze session. On the alternate days between exposure to interference the animals were given sessions on their own maze with the 30sec delay only which was now standard procedure for the rats. The rats were still run on a rotational basis from first through to

fifth within each squad, the inter-trial interval now being 5min due to the extra time required for the 30 delay per trial.

One enriched animal began to perseverate after the maze interference, and never regained adequate performance in the T-maze task at the standard 30sec delay despite extensive training, so it was dropped from the experiment.

Results

Figure 2 shows the data from the initial 12 session acquisition phase. A 2 (Housing: Enriched Vs Isolated) by 6 (Two-session Blocks) ANOVA, with repeated measures on the Blocks factor, confirmed the improvement in performance over sessions (Blocks effect, $F(5, 90) = 9.254$, $P < 0.001$), but there was no Housing effect ($F = 1.07$) or Housing by Blocks interaction ($F = 1.06$). Thus both groups of rats were equally able to acquire the basic T-maze working memory task with a minimum inter-run delay requirement.

Phase 2 involved the introduction of inter-run delays using 0, 30, 60, 90 and 120 second delay periods. Performance for both groups of rats was found to deteriorate across delays (see Figure 3). A 2 (Housing) by 5 (Delay) ANOVA, with repeated measures on the Delay factor, indicates a significant decrease in accuracy across the groups (Delay effect, $F(4,72) = 34.74$, $P<0.001$) with, again, no Housing effect ($F<1.0$) or Housing by Delay interaction ($F<1.0$). Thus the introduction of an inter-run delay period between the information and choice components of the T-Maze task had a substantial effect on working memory, in that the longer the delay the more errors are produced on the choice run.

In the third phase of the experiment the effects of two further forms of manipulation on the basic task, namely explicit interference, were investigated (See Figure 4). With the 30sec delay now standard procedure for the rats, a "Maze Interfernce" and an "Interference Box" were introduced between the information run and the choice run of the basic task.

A 2 (Housing) by 2 (30sec Delay Only Vs Maze Interference) ANOVA, with repeated measures on the interference factor was calculated and the Manipulation effect was highly significant ($F(1,18) = 33.34$, $P<0.001$), indicating that the Maze interference

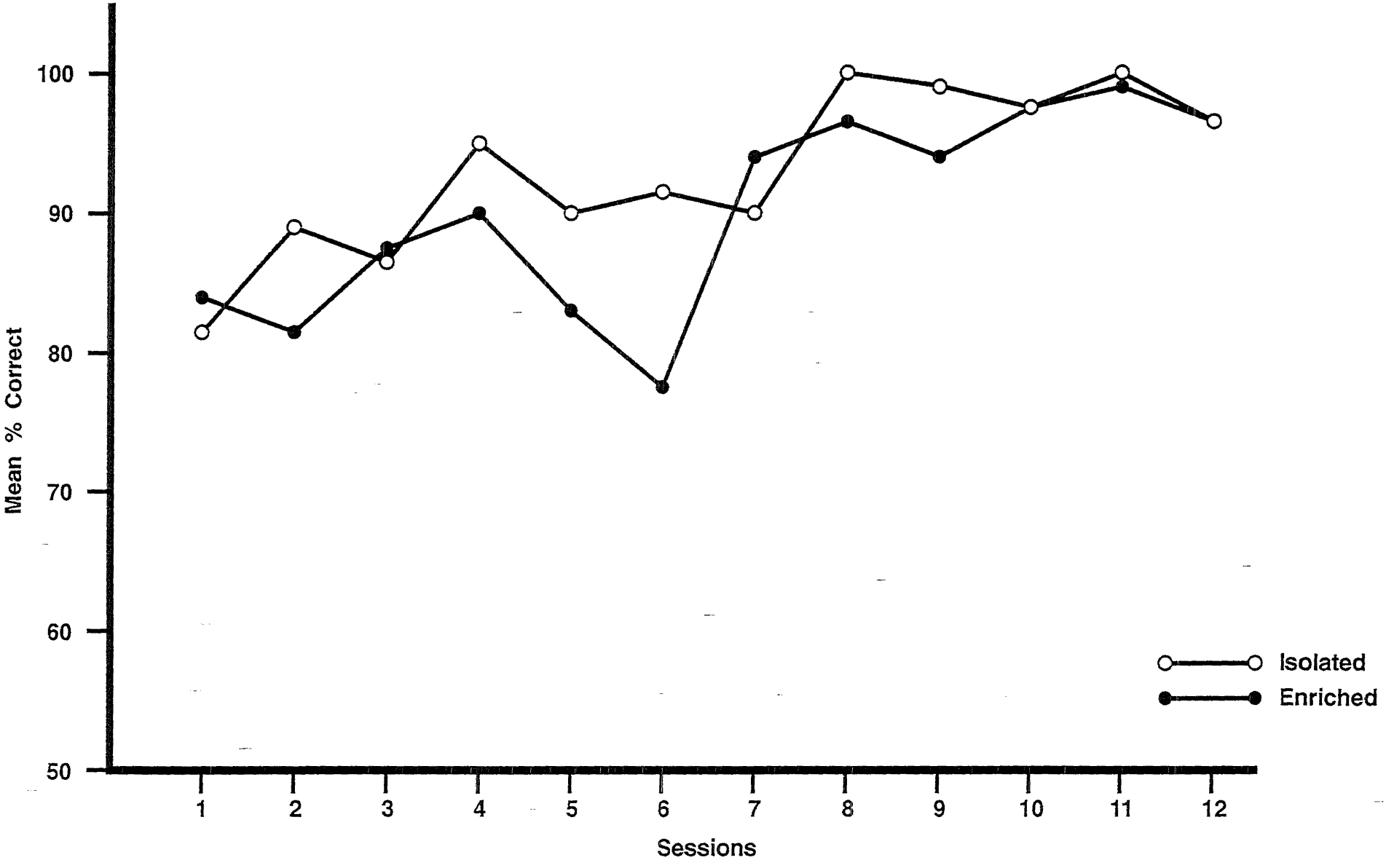

Figure 2 Experiment 1. Mean percent correct scores for isolated and enriched rats on 12 days acquisition of the T-maze matching to sample working memory task.

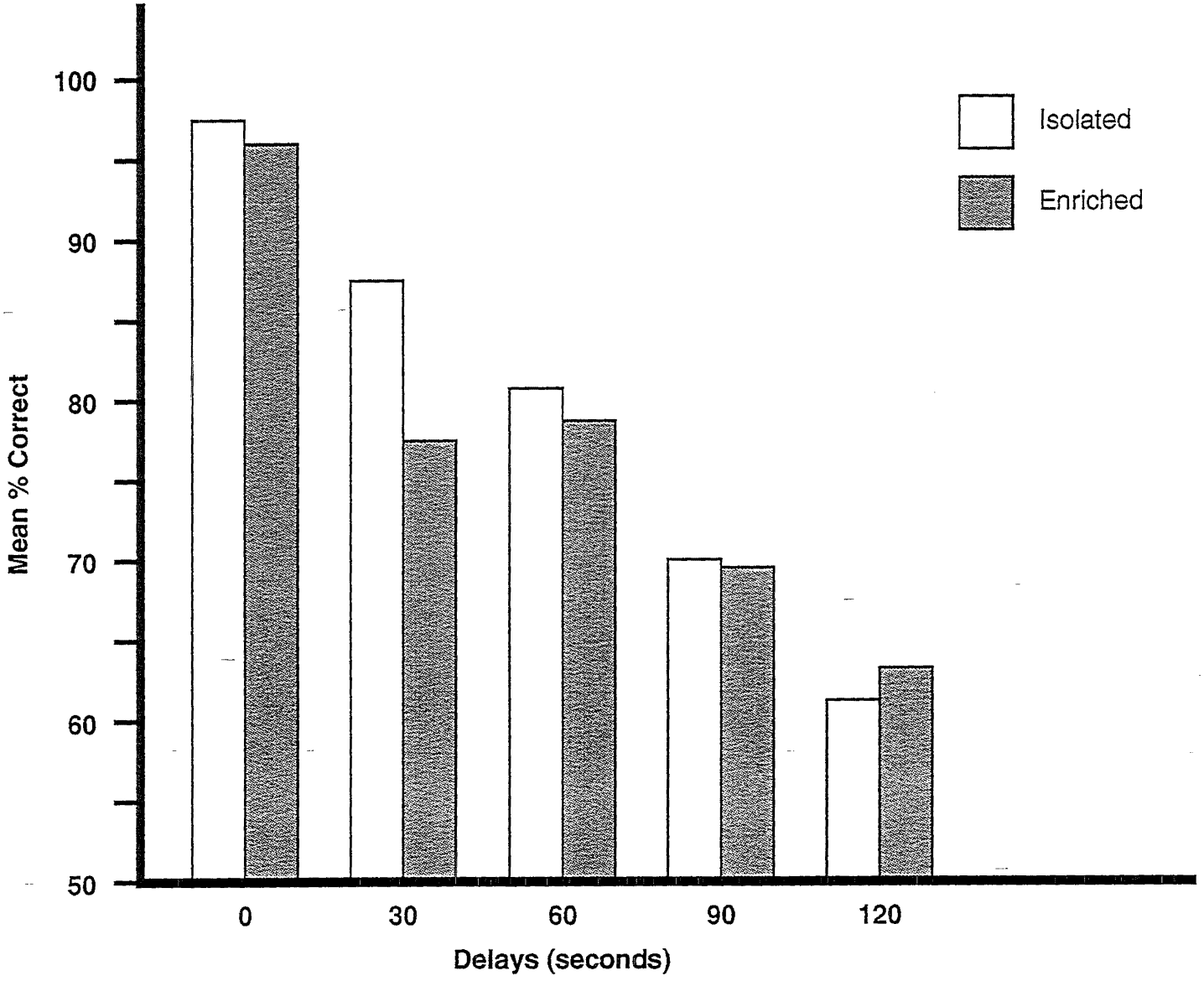

Figure 3 Experiment 1. Mean percentage correct scores for isolated and enriched animals on the T-maze matching-to-sample task at delays of 0, 30, 60, 90, and 120 seconds.

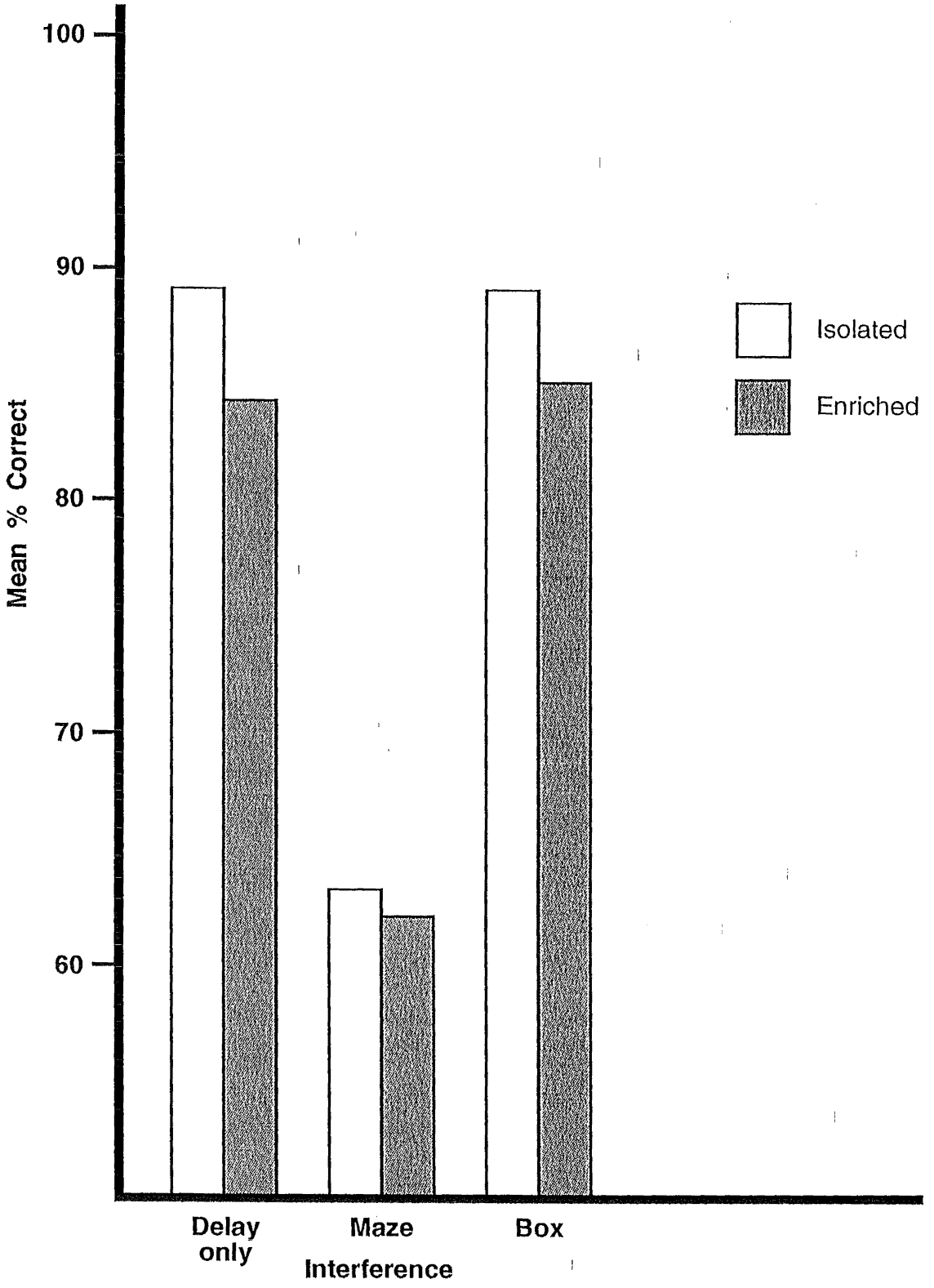

Figure 4 Experiment 1. Mean percent correct scores for isolated and enriched animals on the T-maze matching-to-sample task for maze and box interference with the delay only of 30 seconds.

reduced the accuracy of performance when compared to the 30sec delay only. No Housing or interaction effects were significant ($F<1.0$).

A 2 (Housing) by 2 (Interference: Maze Vs Interference Box) ANOVA, with repeated messures on the interference factor, was also found to be highly significant (Manipulation Effect, $F(1,17) = 22.78$, $P<0.001$) confirming that a 30sec exposure to the Box interference disrupted the task much less than an enforced run on a second maze. Again, no Housing or interaction effects were evident ($F<1.0$).

A final 2 (Housing) by 2 (30sec Delay Only Vs Interference Box) ANOVA with repeated measures on the second factor was conducted, but was not found to be statistically significant ($F(1,17) = 1.46$) indicating that neither the 30sec delay, nor the Box interferer affected performance on the working memory task on either of the two differentially reared groups of animals. No interaction or housing effects emerged ($F<1.5$).

The kinds of object interactive behaviour observed in the Box Interferer was subjected to a 2 (Housing) by 2 (Manipulatory Behaviour Vs Non-manipulatory Behaviour) ANOVA, with repeated measures on the Behaviour factor. A main effect was found for Manipulatory Vs Non-manipulatory Behaviour (Behaviour Effect, $F(1,17) = 669.120$, $P<0.001$). This very significant effect emerged because of the large differences in the frequency of the types of behaviour observed. Manipulatory behaviour had an average of 17 observations per animal per session, whereas Non-manipulatory behaviour, because of the high frequency of Sniffing, had a much higher mean of 67.87 observations per animal per session. No effect of Housing was evident ($F=2.31$).

Sniffing behaviour was then taken out of the Non-manipulatory scores and a further 2 (Housing) by 2 (Manipulatory Vs Non-manipulatory minus Sniffing) ANOVA, with repeated measures on the Behaviour factor, was calculated . The main effect for Behaviour remained highly significant (Behaviour Effect, $F(1,17) = 11.4598$, $P<0.004$), however, it was the Manipulatory Behaviours observed which now exhibited a higher mean frequency (17 observations per session per animal) than the Non-manipulatory minus sniffing Behaviours (12.903 observations per session per animal). An interaction effect for Housing and Behaviour was found to be

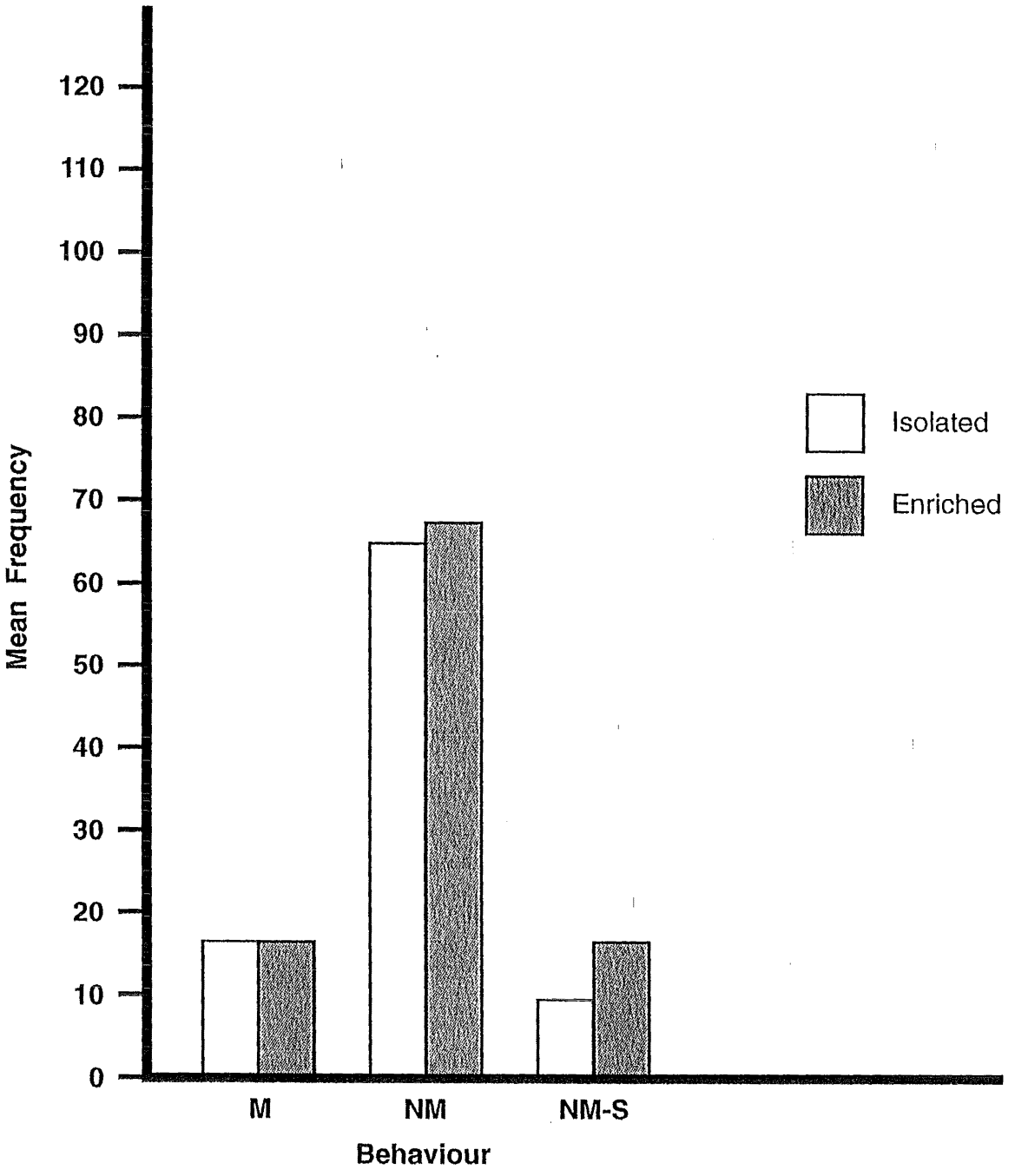

Figure 5 Experiment 1. Mean frequency of Manipulatory (M), Non-Manipulatory (NM) and Non-Manipulatory minus Sniffing (NM-S) behaviour exhibited by isolated, enriched, and long-term enriched animals while in the box interference.

significant (Interaction Effect, $F,(1,17) = 6.7856$, $P<0.02$), and analysis of the simple effects indicated that Manipulatory behaviour was equal over both the IC and EC subjects ($F<1.0$) , but the enriched animals made significantly more Non-manipulatory minus sniffing behaviours than the isolated animals ($F(1,17)=8.39$, $P<0.01$).

Discussion

As was expected the results from the first experiment indicate there was no difference between housing groups in the animals ability to acquire the basic memory task, and there was clear evidence for the various manipulations having an effect on performance on the T-Maze working memory task. Both the delays and the Maze interference had significant effects on performance, and the fact that the Box interference did not would suggest that the nature of the interfering stumulus is of prime importance (Jarrard, 1975).

The fact that differential rearing conditions did not effect the acqusition of the basic T-maze working memory task was consistant with previous research, which found that relatively simple tasks do not yeild significant EC/IC differences in acquisition and performance (Renner & Rosenzweig, 1987). However, the more complex the task, and subsequently increased demand placed on working memory, the more performance differences between EC and IC would be expected to be evident. Differences in performance scores between the two groups should have emerged with the imposition of the series of delays and the two forms of explicit interference, but these expected behavioural differences did not appear.

This could have been for two reasons. Firstly, the animals were not put into their respective environments until 35 days of age. Although this was well within the sensitive period of 25 to 60 days of age (Bennett et al, 1970; Dalrymple-Alford & Benton, 1981; Einon & Morgan, 1978), the pre-exposure to social interaction may have lessened the effect of the isolation for the rats. It is known that some of the behavioural effects of isolation can be inhibited by short

periods of social contact (Einon, Humphreys, Chivers, Field & Naylor, 1981). Perhaps the 10 days of social interaction these animals would have received may have been enough to insulate the rats aginst some of the effects of isolation.

Secondly, the enriched animals were put into and kept in isolation for the entire testing phase; a total period of 56 days which was nearly twice the length of time the animals spent in the enriched environment. Although reversal of housing environment is known not to affect many of the behaviours commonly associated with an enriched rearing condition, such as superior performance in several learning tasks, more subtle effects such as slower emergence latencies have been noted in animals isolated at the time of testing (Dalrymple-Alford & Benton, 1984b). As such, the performance of the enriched animals may have been overshadowed by effects of being isolated at the time of testing and for such an extended duration.

Experiment 2 was designed to overcome some of the problems evident in experiment 1. The rats were be placed in their respective environments at an earlier age, specifically at weaning (20-21 days of age), and a third experimental group was added, namely a long-term enriched group which would remain in their enriched environment throughout the experiment to control for any effects of continued isolation for the duration of the testing phase. In addition, all animals were tested in a standard open field apparatus to examine activity levels between the three experimental groups. This was done to assess whether the characteristic hyperactive behaviour of the Isolated animals would be evident in the other groups due to isolation at the time of testing.

3. EXPERIMENT 2

Method

Subjects:

Thirty Female Albino (Sprague-Dawley) rats were bred and raised in the animal facilities within the department where they remained undisturbed with their dams and siblings until 21 days of age. The animals were then assigned to either permanent isolation (IC), enrichment and then isolation for the duration of the testing phase (EC), or long-term enrichment conditions (LTE) based on litter, age and weight, such that each subject had a comparable littermates in each of the other conditions.

Housing Conditions:

Ten animals were assigned to the isolated condition (IC) which was identical to the conditions used in the first experiment. Another ten rats were assigned to the enriched environment (EC), which again remained identical to the conditions of the first experiment. The remaining ten animals were assigned to the long-term enrichment environment (LTE), during which time this condition was kept as similar as possible to the conditions found in the enriched environment. All animals spent 30 days in their respective conditions before they were all weighed and rehoused in individual cages. The isolated and enriched rats then remained in their individual cages for the remainder of the experiment. The long-term enriched animals, however, remained in their single cages only during the day for testing and feeding, and were regrouped in their enriched environment at night by a neutral party.

A neutral staff member divided the animals into two groups (A & B), each of which had an equal number if isolated, enriched and long-term enriched animals organised in random order such that the testing would be done on a blind basis by the researcher to avoid experimenter bias. The animals were kept in the same room and conditions and the subjects in the first experiment, and remained on free food and water up until the training phase after which the animals were kept at 80-85% of the free feeding weight of animals of the same strain, age and sex.

Apparatus

The same two identical T-mazes were used as in the first experiment.

The open field measured 60 x 60cm with the black floor divided into 16 equal squares; the 30cm high walls were transparent perspex. The field rested 30cm above the floor in the centre of a dimly lit room (20 lx at floor of apparatus) which contained high shelving but no windows or other distinguishing features.

Procedure

Apart from the minor procedural changes noted below, all the training details outlined in Experiment 1 were replicated exactly in Experiment 2. The rats were divided into 5 squads of 6 animals three of which were run on one maze (maze 'A'), and three on the other (maze 'B'). The rats were run on a similar rotational basis as Experiment 1, but from rat one through to six within each squad, with an intertrial interval of 3min.

The measurement of activity in the open field commenced 3 days after the animals were rehoused individually and divided into the squads for running. The rats were placed in the open field for one 6-minute session per day for 5 days. Each session was divided into three 2-minute Blocks, and continuous observation was made of the rats ambulation, rearing, and grooming behaviours. Boli were counted after the rat was removed fron the field.

Phase three which involved the interference component of the experiment had the following alterations. The training of these rats for running on the unfamiliar maze started with the extension of the sessions from eight double-run trials per daily session to twelve double-run trails per session, rather than this extension starting at the time of testing as in Experiment 1. Only one days training was then given on the unfamiliar maze to accustom the animals to the unfamiliar smells etc., and two days instead of three were run with the 30 second delay as the standard interval between presentation and choice component of the trial. This was done because these animals had reached the same level of performance of the animals in experiment 1.

The objects in the "Interference Box" were identical, used in the same sequence and placed in the same positions within the box as in the first experiment.

At the completion of the experiment the rats were anaesthetised with Pentobarbital, then perfused through the heart with saline, then with 4% formalin. The brains were removed then weighed on a Mettler H30 scale to the nearest milligram.

Results

Analysis of the animals activity in the open field comprised of the three behaviours observed, namely Ambulation, Rearing, and Grooming.

Ambulation

A 3 (Housing) by 5 (Days: Day 1-5) by 3 (2-Minute Blocks) ANOVA, with repeated measures on the Days and Minute factors, was performed on the Ambulation data. A significant Days effect emerged (Days Effect: $F(4,108) = 35.36$, $P<0.001$), as well as a significant effect for the Blocks of Minutes (Blocks Effect: $F(2,54) = 72.23$, $P<0.001$), but no Housing effect was evident ($F = 1.2$). This would suggest that the rats Ambulation activity changed not only across the 5 days, but also within each day.

Three interaction effects were evident. The first was an interaction between the Housing Condition and Blocks of Minutes (Housing by Blocks Interaction: $F(4,54) = 3.22$, $P<0.02$), (see Figure 5). An examination of the means for the three housing groups over the three blocks per day indicated that the Isolated animals remained quite active over the blocks within each day. The Enriched rats had an elevated initial block activity but habituate quite quickly and their activity decreases over the blocks within each day. The Long-term Enriched animals were very similar to the IC animals in their initial activity scores for Ambulation, but they habituated more quickly and to a greater extent than either of the other groups of rats, with an overall lower ambulation score in the final time Block.

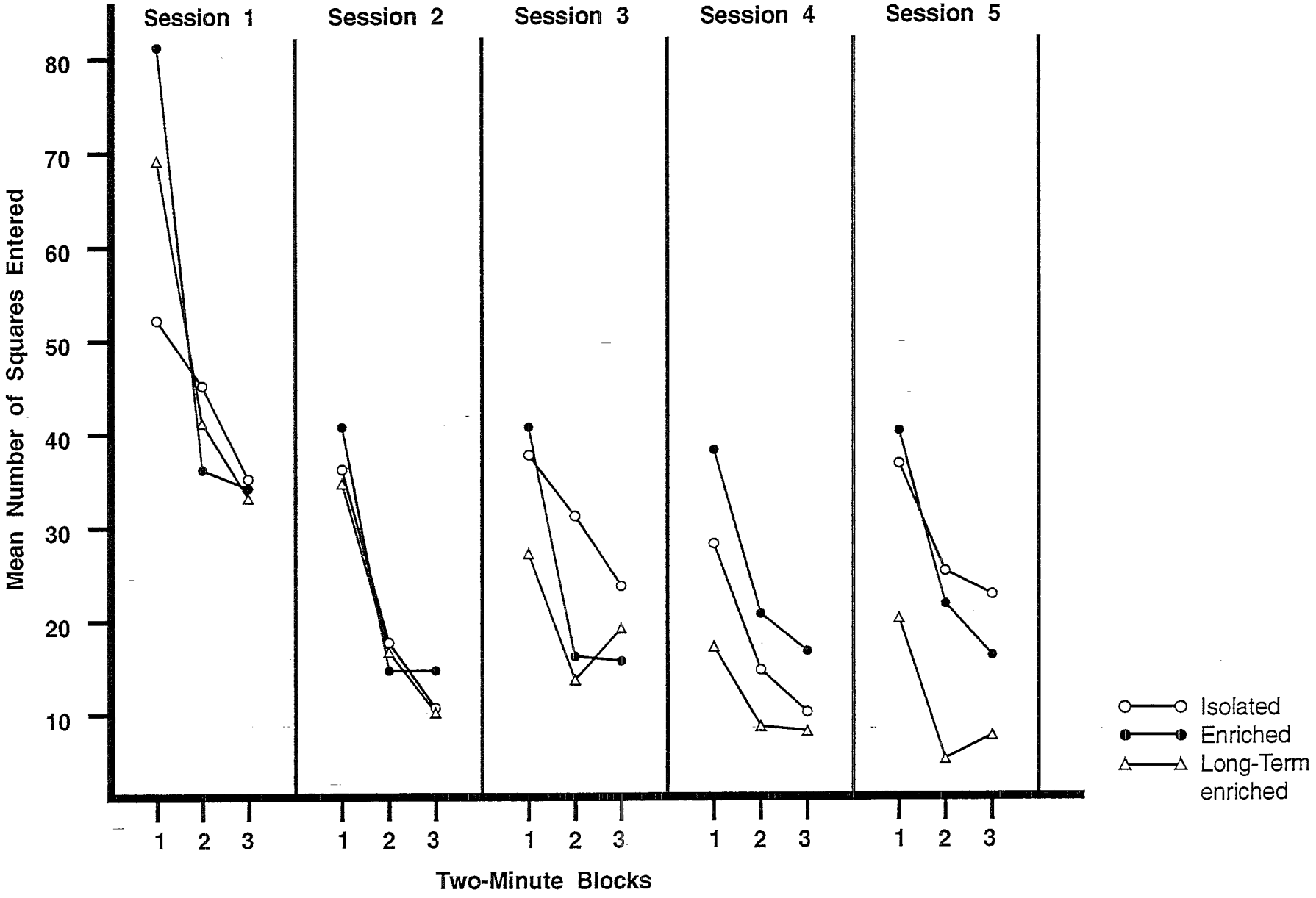

Figure 6 Experiment 2. Mean ambulation frequency in an open field for isolated, enriched, and long-term enriched animals across the three 2-minute blocks over 5 daily sessions.

A Days by Minutes interaction was also highly significant (Days by Minutes Interaction : $F(8,216) = 4.09$, $P<0.001$), and is illustrated in Figure 6. Day 1 has a much higher level of ambulation than the other 4 days, but it also has the largest decrease over the blocks of minutes. This may have been emphasised, however, by the comparitively lower level of ambulation which is maintained over days 2 to 5 in comparason to the high level in day 1.

The three-way interaction between Housing, Days and Blocks of Minutes was also significant (Housing by Days by Blocks Interaction Effect: $F(16,216) = 1.73$, $P<0.05$). As can be seen in Figure 5 all groups of rats can be seen to habituate over days, and also within each day, although the IC animals exhibit this to a lesser degree than the other groups. The EC animals appear much like the IC rats in their first block on every day, but tend to habituate more quickly to a lower general level of activity than the isolated rats. The LTE group have an intermediate level of initial ambulation compared to the other animals, but they habituate more quickly than either of the other housing groups, and fall to a much lower lever than is exhibited by the other animals.

A Housing by Days interaction failed to reach an acceptable significance level ($F(1,108) = 1.91$, $P<0.07$).

Rearing

Analysis of the Rearing behaviour in the open field was completed in an identical manner to the ambulation with a 3 (Housing) by 5 (Days) by 3 (2-Minute Blocks) ANOVA with repeated measures on the Day and Block factors. The Days factor was highly significant (Days Effect: $F(4,108) = 17.69$, $P<0.001$), and from Figure 7 it can be seen that the first day was the most active for rearing with a pronounced decrease on the second day, after which the decline continued in smaller steps. A Housing by Days interaction effect was also evident (Housing by Days Interaction Effect: $F(8,108) = 2.55$, $P<0.02$). Figure 7 illustrates that the Isolated animals maintained their rearing behaviour across the 5 days with almost no decrease in frequency, whereas both the Enriched and the Long-term Enriched show a decrease from the second exposure to the open field which continues to decline. The LTE group did, however, have a slightly higher initial frequency of rearing, and their rearing on the last trial was considerably lower than the other two groups.

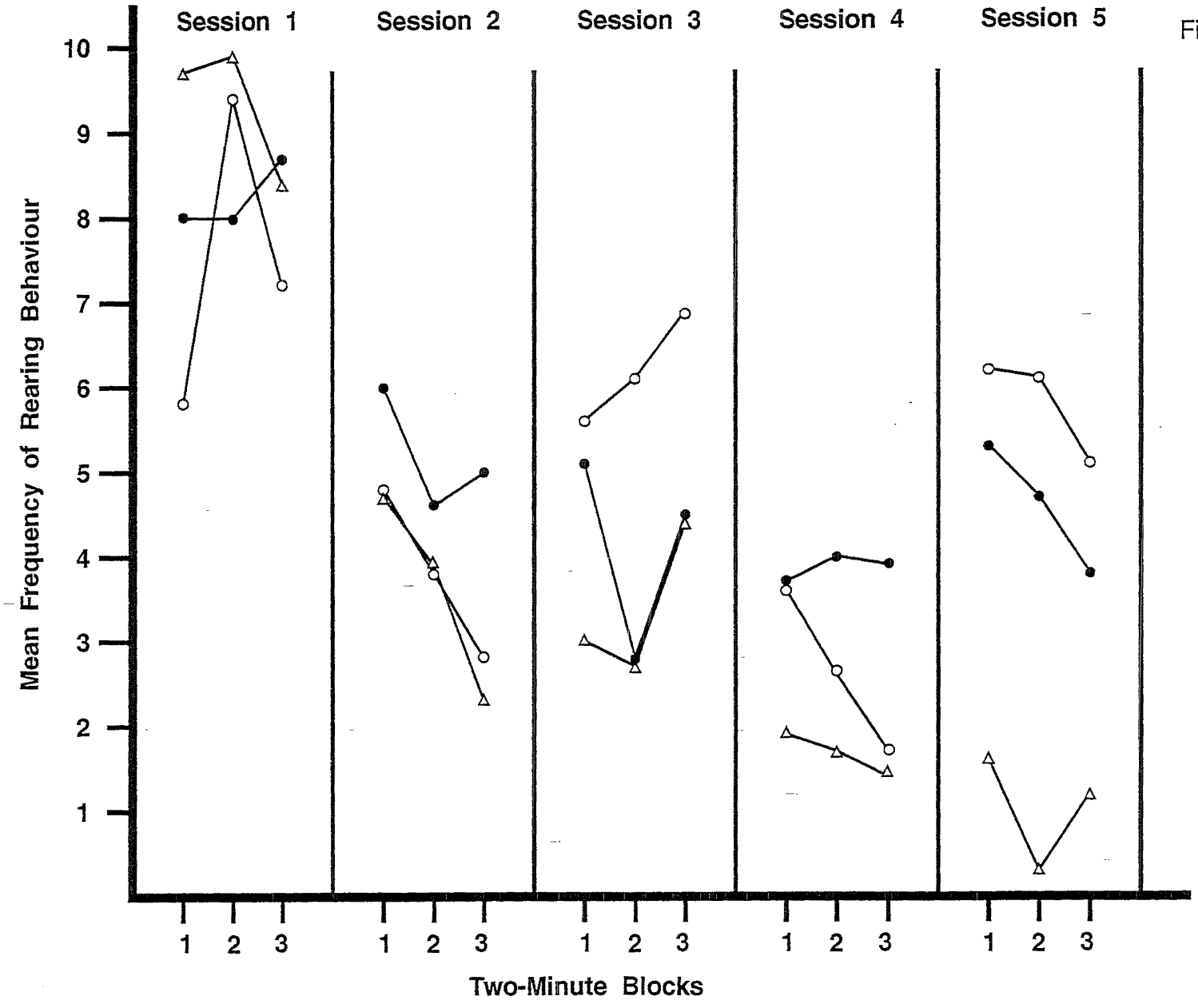

Figure 7 Experiment 2. Mean frequency of rearing behaviour observed in isolated, enriched, and long-term enriched subjects across the three 2-minute blocks over 5 daily sessions.

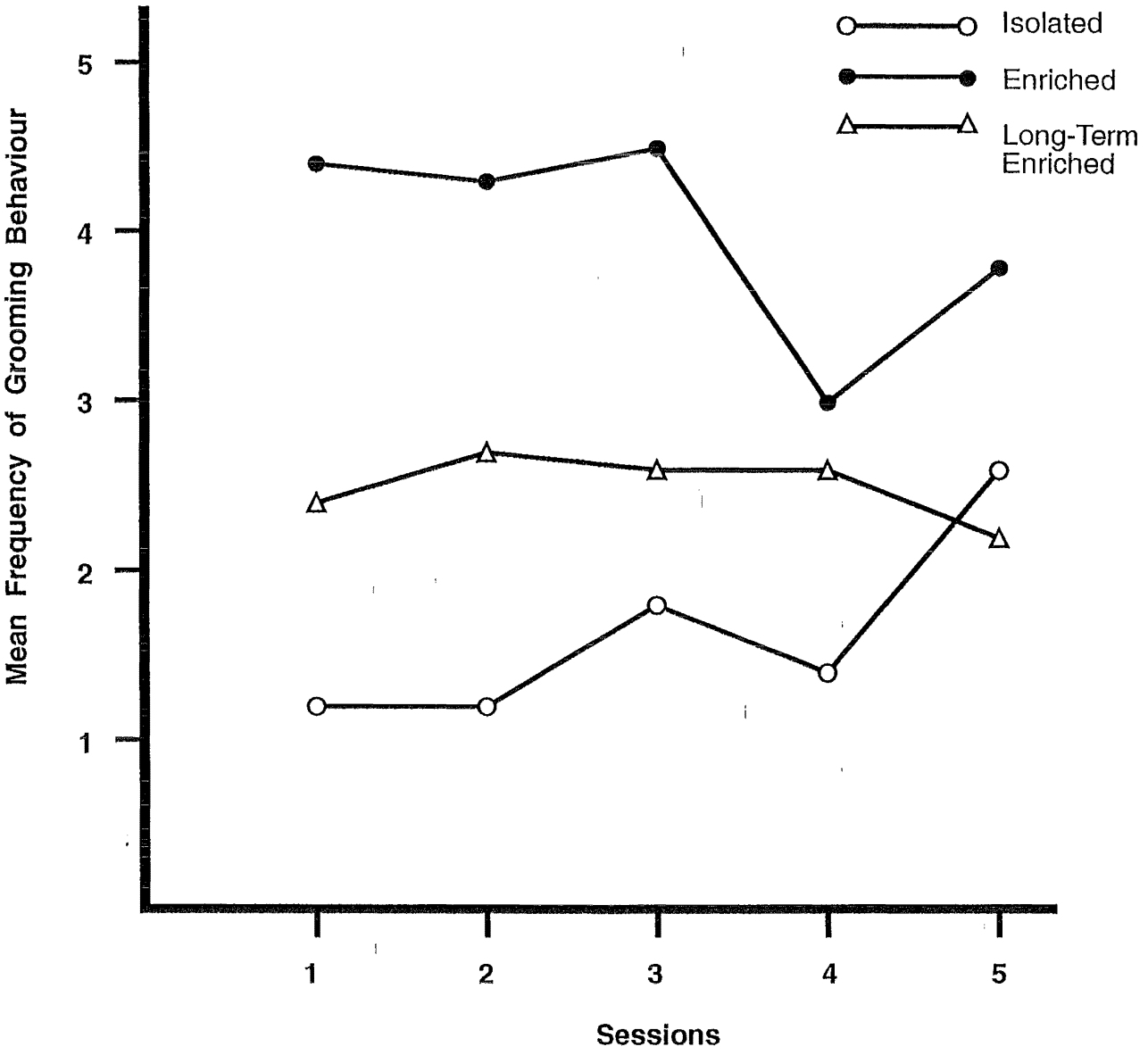

Figure 8 Experiment 2. Mean frequency of grooming behaviours observed in isolated, enriched and long-term enriched animals over five daily 6 minute sessions in an open field.

A second interaction effect was also evident between Days and Minutes (Days by Minutes Interaction Effect: $(F(8,216) = 2.14, P<0.04)$. This significant effect appears to be caused by fluctuations in rearing behaviour on days one and three by the isolated animals.

Grooming

A 3 (Housing) by 5 (Days) by 3 (2-Minute Blocks) ANOVA, with repeated measures on the Days and Blocks factors, was performed on the grooming data and a significant effect for Housing emerged (Housing Effect: $F(2,27) = 10.59, P<0.001$). Post-hoc analysis indicated a difference in grooming frequency between IC and EC rats ($F(1,27) = 20.67, P<0.001$) and between EC and LTE animals ($F(1,27) = 8.33, P<0.01$), but not between IC and LTE ($F = 1.23$) (see Figure 8). No other main or interaction effects were found ($F<1.55$).

Figure 9 shows the data from the initial 12 session acquisition phase (3 sec presentation-choice interval). A 3 (Housing: Isolated vs Enriched vs Long-term Enriched) by 6 (Two-session blocks) ANOVA, with repeated measures on the Blocks factor, confirms an improvement in performance over sessions (Blocks effect, $F(5,135) = 8.139, P< 0.001$), but there was no effect for Housing ($F = 1.24$), nor was there a Housing by Blocks interaction ($F = 1.27$). In the first session there was a strong suggestion of a Housing effect, so a one-way Levels of Housing (3) ANOVA was then conducted but it failed to reach significance (Session Effect, $F(2, 27) = 2.89, P<0.07$). Thus, all three groups of rats were equally able to acquire the basic T-maze working memory task with a minimum delay requirement.

Phase 2 of the experiment involved the introduction of the series of time delays between presentation and choice. These data were subjected to a 3 (Housing) by 5 (Delay: 0, 30, 60, 90 & 120 Seconds) ANOVA, with repeated measures on the delay factor, and this confirmed that performance deteriorated a significant degree the longer the delay (Delay Effect, $F(4, 108) = 33.93, P<0.001$). Post-hoc analysis was done to accertain whether some delay periods were more disruptive than others to the rat's performance on the memory task. The most significant disruption to performance appeared at the very first delay of 30 seconds ($F(1,27) = 34.64, P<0.001$), with no significant change of performance between the 30 sec to 60 sec ($F(1,27) = 1.36$), but then further disruption in the performance from 60sec to 90sec ($F(1,27) = 10.23, P<0.004$), then no further significant

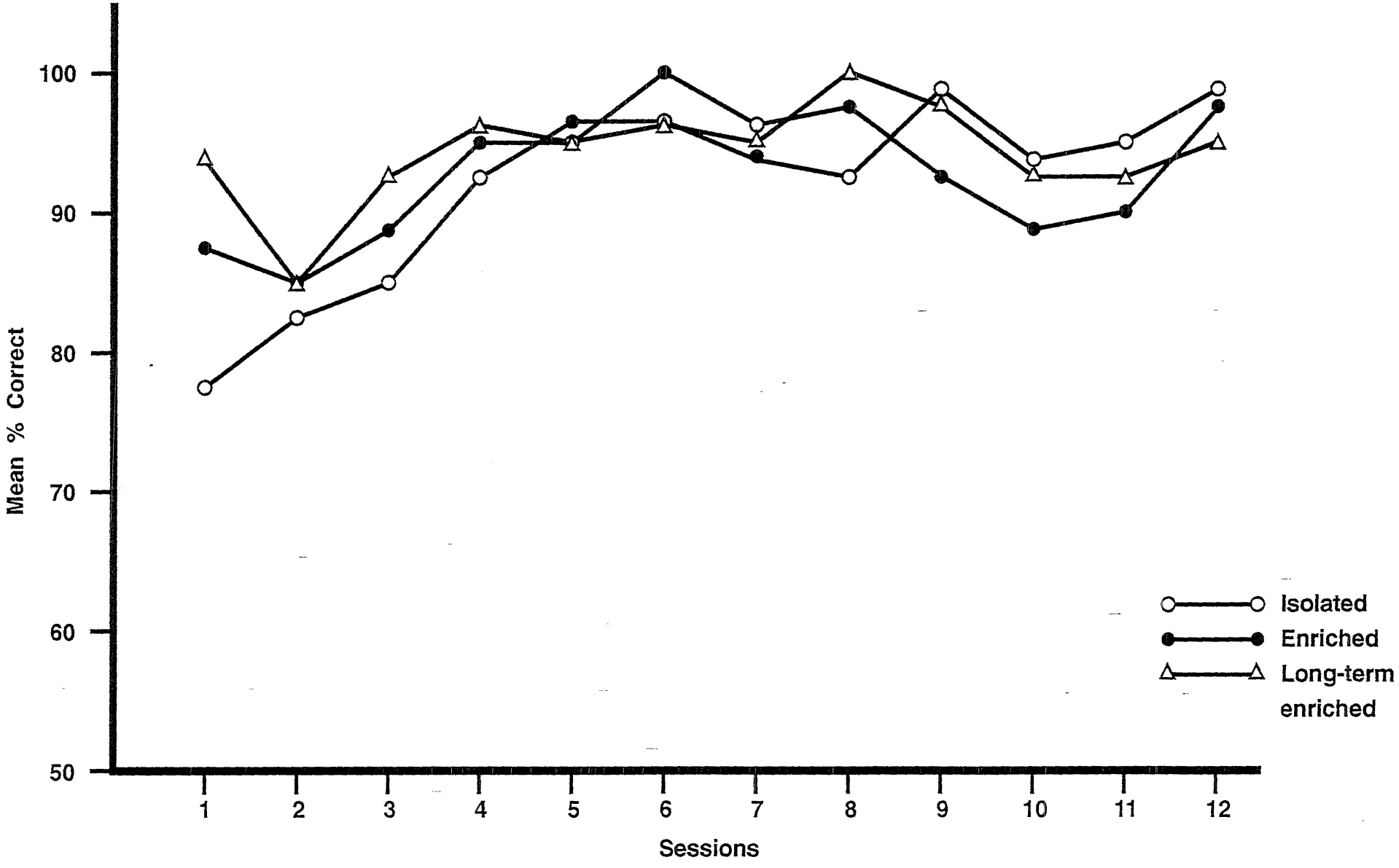

Figure 9 Experiment 2. Mean percent correct scores for isolated, enriched, and long-term enriched rats on the 12 session acquisition of the T-maze matching-to-sample working memory task.

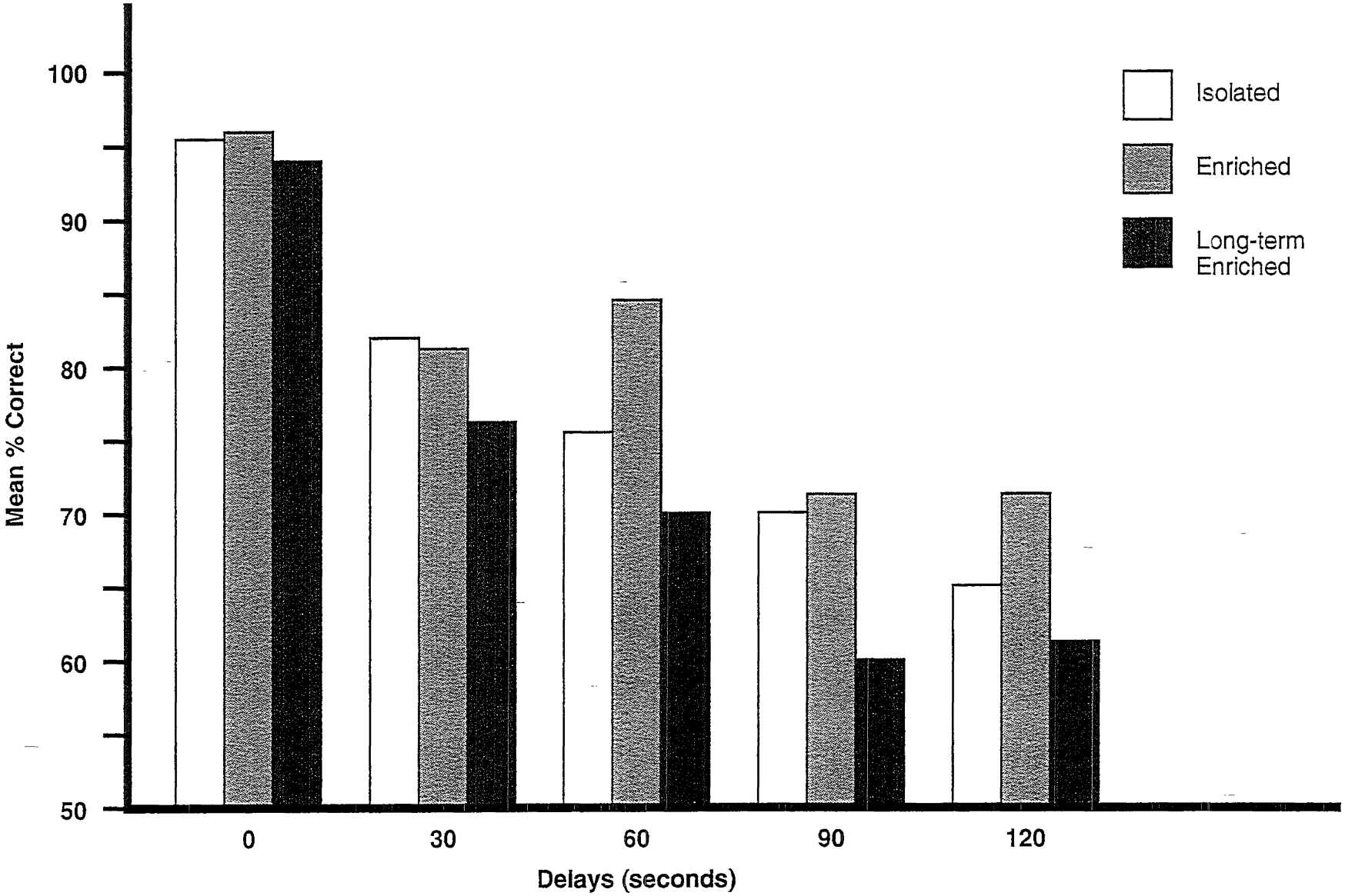

Figure 10 Experiment 2. Mean percent correct scores for isolated, enriched, and long-term enriched animals on the T-maze matching to sample tasks at delays of 0, 30, 60, 90, and 120 seconds.

disruption from 90 to 120 sec ($F<1.00$). Hence, the initial exposure to a delay appears to impact upon performance of the basic memory task the most, only to plateau at the next delay period to then drop again at the exposure to the 90sec delay period, to then again plateau at 120sec.

Although a main effect for Housing failed to reach significance levels (Housing Effect, $F(2,27) = 3.13$. $P<0.06$), the result was close, so pairwise comparisons were investigated for the 5 Delay periods (0'sec delay - 120'sec delay). A significant difference in performance between the Enriched animals and the Long-term Enriched ($F(1,27) = 6.51$, $P<0.02$) was evident, but not between the Isolates and Long-term Enriched ($F = 2.5$), nor between the Isolates and Enriched ($F<1.0$). Hence, the performance of the EC rats was least effected by the delays, the LTE rats were most effected, with the IC animals performance in between (see Figure 10).

Due to this suggestion of a Housing effect the delay data were then subjected to further analysis excluding the 0-delay scores in a 3 (Housing) by 4 (Delay: 30, 60, 90 & 120 sec) ANOVA, with repeated measures on the Delay factor. A main effect for Housing (Housing Effect: $F(2,27) = 3.34$, $P<0.05$) was evident, and analysis of the means revealed a significant difference in peformance between the Enriched and Long-term enriched subjects ($F(1,27) = 6.56$, $P<0.02$), but no differences between Isolated and Enriched ($F<1.0$), nor between Isolated and Long-term enriched ($F = 2.48$). No interaction effect between Housing and the 4 delays emerged ($F<1.0$).

In the third phase of the experiment the effects of two forms of interference upon the basic T-maze memory task were investigated. (see Figure 11) with each interferer being separately analysed against the 30sec Delay Only, and then together. A 3 (Housing) by 2 (Delay Only vs Box Interference) ANOVA, with repeated measures on the second factor, yeilded a significant effect for Interference (Box Interference Effect: $F(1,27) = 12.10$, $P<0.003$). No Housing ($F<1.0$) or interaction effects ($F<1.0$) were evident.

The 3 (Housing) by 2 (Delay Only vs Maze Interference) ANOVA, with repeated measures on the second factor, also yeilded a significant effect (Maze Interference Effect: $F(1,27) = 48.89$, $P<0.001$), but no Housing ($F<1.0$) or interaction effects ($F = 1.85$). The third 3 (Housing) by 2(Box vs Maze) ANOVA, with repeated measures

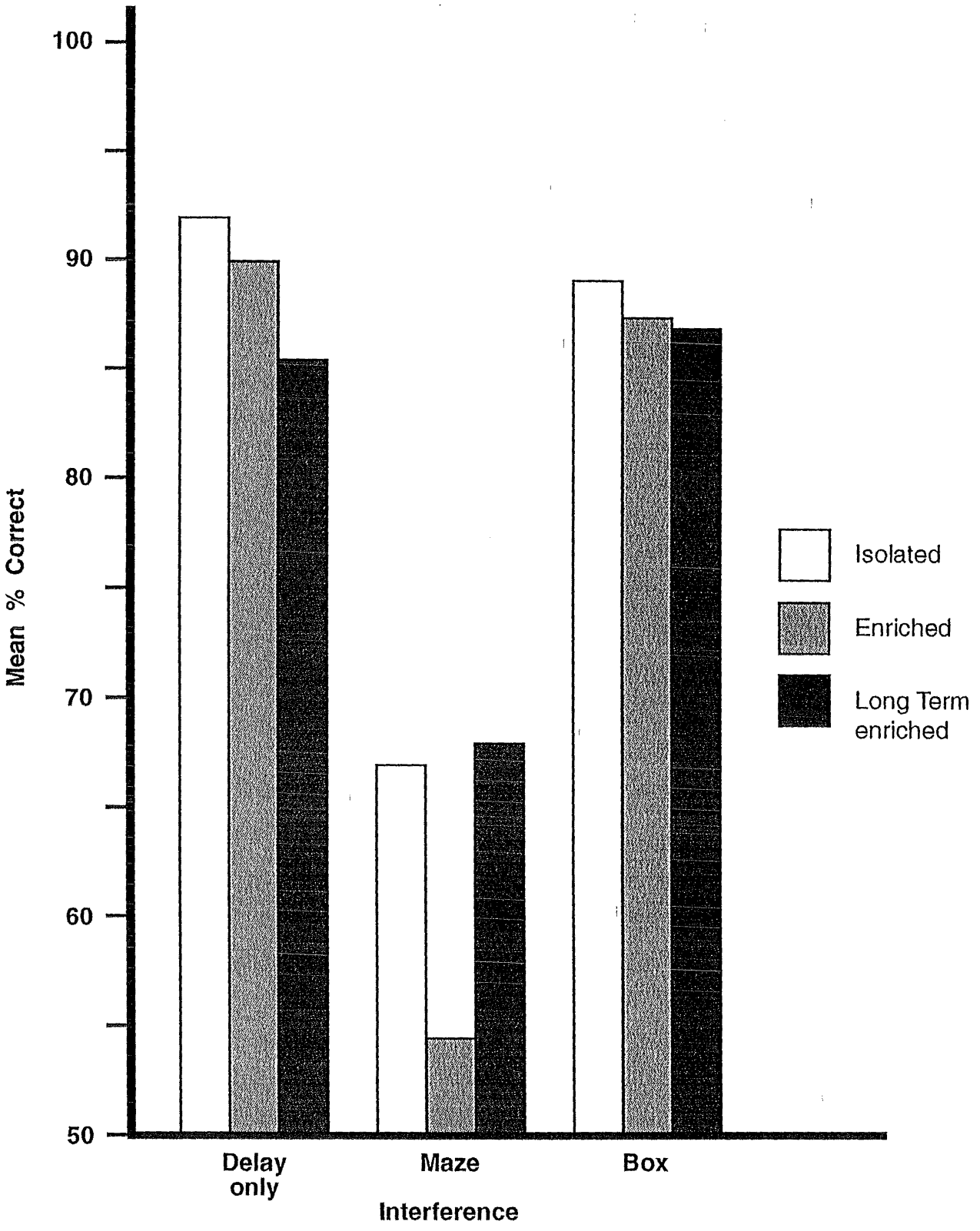

Figure 11 Experiment 2. Mean percent correct scores for isolated, enriched, and long-term enriched rats on the T-Maze matching-to-sample task for the Maze and Box interference with the delay only of 30 seconds duration.

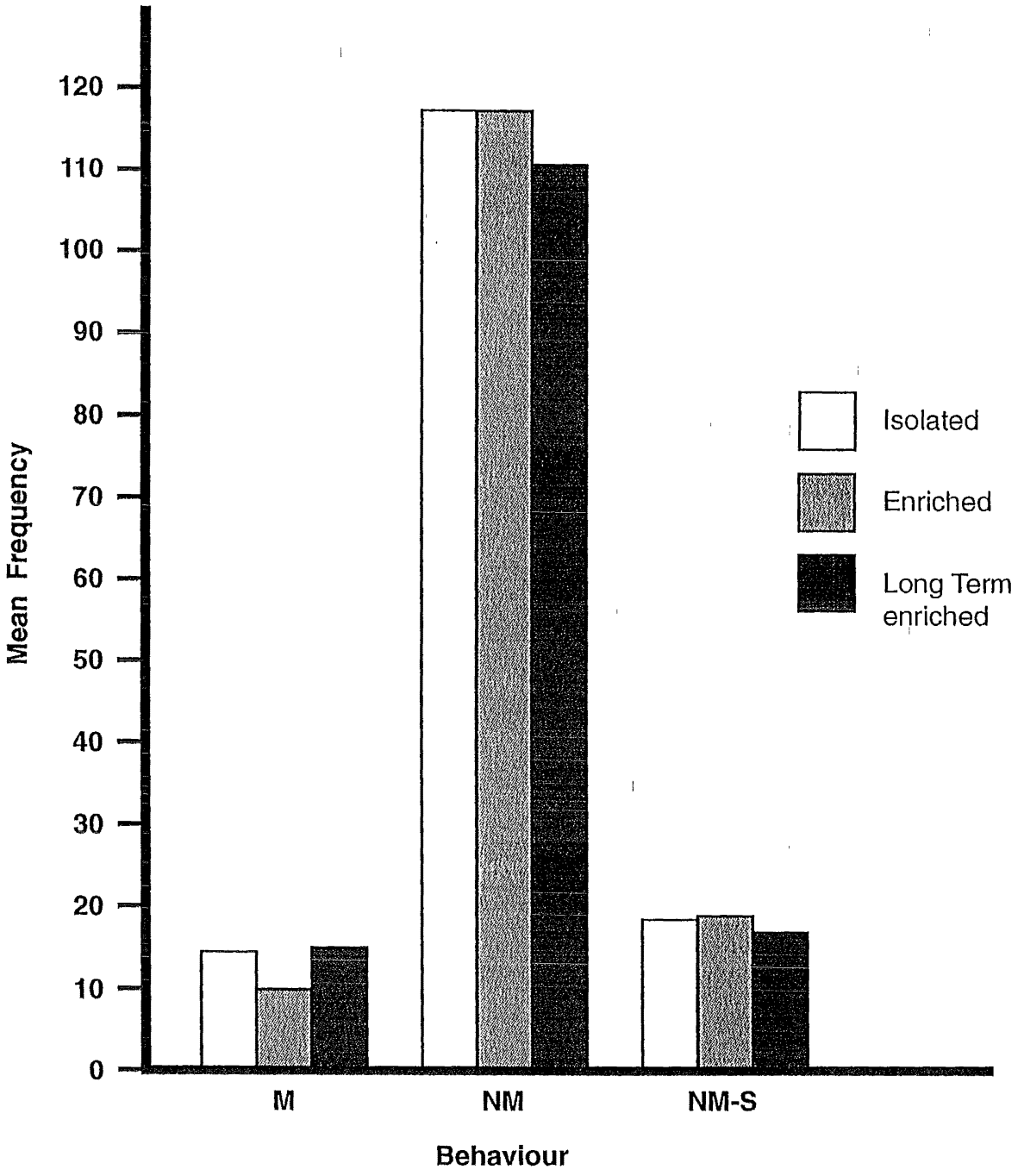

Figure 12 Experiment 2. Mean frequency of Manipulatory (M), Non-Manipulatory (NM), and Non-Manipulatory minus Sniffing (NM-S) behaviour exhibited by isolated, enriched, and long-term enriched animals while in the box interference.

on the second factor, yeilded similar results with a significant effect for the interference ($F(1,27) = 19.03$, $P<0.001$), but no Housing ($F<1.0$) or interaction ($F<1.0$) effects. In summary, the performance on the T-maze working memory task with the 30 second delay only was significantly affected by both the Maze and Box interference. However, as Figure 11 illustrates, Maze interferance disrupts performance more than the Box interference.

While the rats were in the Box Interference the nature and frequency of their interactions with the objects were recorded (see Figure 12). A 3 (Housing) by 2 (Manipulartory vs Non-Manipulatory Behaviour) ANOVA, with repeated measures on the Behaviour factor, was calculated, and a significant effect for Manipulartory vs Non-Manipulatory Behaviour was exhibited (Behaviour Effect: $F(1,27) = 1451.27$, $P<0.001$). No significant effects for Housing was found ($F<1.0$), nor was there any interaction between the factors ($F<1.0$).

A closer look at the reason behind the large effect for exhibited behavior revealed a large discrepancy between the amount of Manipulatory Behavior (averaged across groups = 13.23) and Non-Manipulatory Behaviour (averaged across groups = 115.33). This was found because sniffing, which is Non-Manipulatory, composed the majority of the behaviour in this section. An analysis of the behaviours without the sniffing component was then completed with a 3 (Housing) by 2 (Manipulatory vs Non-Manipulatory minus Sniffing Behaviour), with repeated measures on the Behaviour factor. The Manipulatory vs Non-Manipulatory minus sniffing comparason continued to be highly significant (Behaviour Effect: $F(1,27) = 16.2$, $P<0.001$) confirming that even without the sniffing component the frequency of Non-Manipulatory minus sniffing (mean = 18.06) behaviours was higher than Manipulatory (mean = 13.23) behaviours for all rats. Although the interaction between Housing and Behaviour failed to reach significance (Interaction Effect: $F(2,27) = 2.87$, $P<0.08$) it warranted a closer inspection of the means, and it appears that the EC animals displayed less manipulatory behaviour than the rats in either of the other housing conditions.

The gross weight of the rat's brains were analysed using a one way Levels of Housing (3) ANOVA which failed to reach a satisfactory level of significance ($F<1.0$). However, standard deviations indicate that the LTE gross brain weights showed less variation (629.81) than did the Enriched (580.70) and the Isolated (399.63) groups.

4. GENERAL DISCUSSION

All the animal subjects over the two experiments were equally able to acquire the basic T-maze nonmatching-to-sample working memory task. This result is consistent with the previous literature which suggests that with relatively simple tasks performance differences between animals reared in differing environments would not be evident. However, the more complex the task, the more likely that performance differences between the differentially reared animals would be expected to emerge (Renner & Rosenzweig, 1987).

The first manipulation to test retention on the t-maze working memory task was the introduction of a series of brief delays. Previous research using delays of similar duration found that performance scores deteriorate the longer the delay. Tonkiss, Feldon & Rawlins (1990) found increased errors with a delay of 20 seconds, as has Dunnett (1990), Dunnett et al (1988), and Gordon et al, (1976). Aggleton, Hunt & Rawlins (1986) found that a delay of 20 seconds duration had no effect on performance, but a longer duration of 60 seconds did. Longer delays of 2 to 4 minutes have, likewise, produced effects on the accuracy of performance in behavioural tasks (Jarrad, 1975; Stanton et al, 1984). This effect of brief delays on performance, however, would be expected to be dependant on the apparatus used, and on nature of the behavioural task.

The Radial maze was used by Bolhuis et al (1986) to test spatial working memory, but delays of 5, 20, 60, 120 and 240 minutes were required to produce a decrement in behavioural accuracy, which were much longer than the brief delays used in this research. However, a similar pattern was evident in that the longer the delay the more errors were made. Dunnett (1990) and Dunnet et al (1988) used operant chambers to examine matching and nonmatching-to-sample contingencies with brief delays up to 24 seconds in duration and found that the longer the delay, the more behaviour was affected. Y-maze alternation tasks were used by Aggleton et al (1986) and Jarrard (1975), and both researchers found that delays up to 60 seconds had significant effects on their subjects performance. Researchers using the T-maze apparatus to test

working memory retention have incorpoated various delay durations from 20 seconds (Gordon, Brennan & Schlesinger, 1976; Stanton et al, 1984; Tonkiss et al, 1990) to 10 minutes (Hepler et al, 1985) and the effects of duration on the rats performance reveal the same pattern of responding as the other research using delays; the longer the duration the more disruption to performance.

Due to the recurring pattern of results in the literature it was expected that performance scores would deteriorate as the delay duration got longer, and the results confirmed that brief delays up to 120 seconds decreased the accuracy of performance across all groups of subjects over both experiments. This decrease in accuracy indicated that the incorporation of delays into the basic task made the task more difficult for the animals, therefore, if any differences in memorial capacity existed between the differentially reared animals then it should be apparent here. Experiment 1 found no difference between the performance of the EC and IC rats, but this could have resulted because the animals were not differentially housed until 35 days of age, and this early exposure to social interaction may have decreased the impact of the rearing environments upon behaviour, especially for the animals which were put into permanent isolation.

In Experiment 2 all subjects were differentially housed at the age of 21 days, and the performance on the T-maze task across all experimental groups was affected by the imposition of the delays as was expected. Although no Housing effects were significant, there was the suggestion of a difference, and further analysis revealed that the Long-term enriched animals were more affected by the delays than the Isolated subjects, and the Enriched animals were affected least of all. Renner & Rosenzweig (1987) have suggested that animals exposed to an enriching rearing environment exhibit a superior cognitive and memorial capacity than isolated animals, therefore enriched animals would be less affected by any behavioural manipulation intended to disrupt performance on a simple memory task. As such, this direction of the housing result was contrary to what was expected.

This assumption of superior cognitive ability in Enriched animals is derived from results gained in some learning situations, and although enriching environments have been found to produce superior performance in tasks such as in the Hebb-Williams maze (Cummins et al, 1973, Dalrymple-Alford & Benton, 1984b; Dell & Rose, 1986; Murtha et al, 1990), this may not be an indication of increased memorial capacity. This superior effect in enriched animals can be accounted for, at least in part, by a greater response flexibility inherent in the enriched subjects. The Hebb-Williams maze involves the presentation of a series of problems (Renner & Rosenzweig, 1987), as do reversal problems and motor transfer tests. In all cases, the enriched subjects make fewer errors and solve more problems than their isolated counterparts (Einon et al, 1978; Renner & Rosenzweig, 1987). But these tasks are not a pure indication of memory ability, but also include an indication of the animals ability to alter their response strategy in response to the changing nature of the task.

The radial maze is also considered to be a test of working spatial memory, and yet Einon (1980) has found differences between enriched and isolated animals in the response strategies employed to solve an 8 arm radial maze. In this type of apparatus the animals have to retain information regarding their own position relative to the 8 goal points, and information about the position of the goal points relative to maze's position within the room. Therefore, the radial maze spatial working memory task is more than an indication of spatial working memory ability; it also involves aspects of response learning, visual discrimination, and possibily scent marking discrimination (Einon, 1980). As such, any differences in performance between enriched and isolated animals in a radial maze may not be the result of differing memory or cognitive capacity, but due to any one, or a combination of the different performance variables associated with a radial maze.

The same conclusion can be applied to the Hebb-Williams apparatus; it is not a pure test of memory capacity, but a measure of many interrelated variables. The fact that enriched animals have superior performance on this task above that produced by isolated

animals is not under dispute, but this behavioural difference is not soley due to an enrichment-induced increase in cognitive or memorial capacity. In order to test for differences in working memory a pure task, or as pure as possible task, of working memory is required. The T-maze working memory task employed in this thesis did not involve any requirement of the rats to alter the nature of their response, all they had to do was to specifically remember where they had last been from a choice of two goal points. As such, this task is seen as a pure indication of spatial working memory ability, as any differences in response strategy would not be evident.

If any cognitive differences were to exist between the Isolated, Enriched and Long-term Enriched subjects in this experiment then it should emerge in the animals performance when delays are imposed in the working memory task, and should be in the direction of the LTE animals exhibiting less impairment than the IC animals with the EC subjects in between (Einon, 1980; Einon et al, 1980). But, the results indicate that the LTE animals exhibited poorer performance than the EC housing group, and less accuracy than the IC animals, which is contrary to the notion that working memory performance in Isolated animals is inferior.

The difference in housing conditions between the EC and LTE animals at the time of testing may suggest a reason behind this discrepant result. Both EC and LTE groups had identical housing conditions up until the time of behavioural testing, after which the EC animals were placed into total isolation, and the LTE were placed into isolation only during the day for tesing purposes then put back into the enriched environment at night. In other words, this equates to a partial-enrichment condition. Considering that this is the only difference between the two groups, it can only be assumed that any behavioural differences may result as function of the nature of this housing, although why this partial-enrichment would result in poorer performance by these animals remains unclear. Poorer performance during the delay may result from interfering events during the delay period, and analysis of the results when exposed to differing forms of interference may shed some light on the behavioural differences between the experimental groups.

The introduction of two forms of explicit interference, yeilded similar results across both experiments despite the age of differential exposure being varied across the two experiments; the Maze Interference affected performance more than the Interference Box, relative to the Delay only. In both experiments exposure to the "Maze Interference" was less than 10 seconds in duration, and placed at the beginning of the 30 second delay, whereas exposure to the "Interference Box" was for the entire 30 second duration. This would suggest that it is the nature of the intervening task which is of prime importance, and not the duration of the interference or the placement of the interference within the delay period (Jarrad, 1975; Tran & Beatty, 1985). The Maze interference involved giving the rats conflicting information on the alternate maze, whereas the Interference Box did not provide any Maze-like information or cues. In Experiment 2 exposure to the Interference Box did affect performance. As such, analysis of behaviours during the Interference Box is required.

Object interactions within the "Interference Box" showed a significant difference between Manipulatory and Nonmanipulatory kinds of behaviour across both experiments. In Experiment 1, when sniffing behaviour was taken away from the Nonmanipularory figures, it was the Manipulatory behaviours which were slightly more frequent, whereas in Experiment 2, the opposite was the case, with Nonmanipulatory behaviours maintaining a slightly higher frequency. The most significant difference between the two experiments is in the difference in overall activity in the "Box Interference". The animals in the second experiment exhibited nearly twice the amount of Nonmanipulatory behaviour within the same period of time. Perhaps this explains why the Box interference had a significant effect on performance in the second experiment, and not in the first; the animals were more occupied within the same delay period of time.

Procedural differences between the two experiments may account for some of this observed difference in Non-manipularoty behaviours. In Experiment 1 the subjects were 146 days of age at the

time of the final Interference Box test, whereas the animals in Experiment 2 were 127 days of age at the same stage of testing. Given the extent of behavioural training and testing, it is thought that this age difference between the two experimental groups is not large enough to cause such significant differences in behaviour. A second difference between the two experiments is the open field testing the Experiment 2 animals were subjected to which may have lessened any neophobic reaction to the Interference Box. If this previous exposure to a novel situation did have an effect, it would be most likely to appear in the form of Housing effects, where the types of behaviours observed would reflect those found in the literature with differences in object contact frequencies between the Isolated and LTE animals (Dalrymple-Alford & Benton, 1984b; Einon & Morgan, 1976; Widman & Rosellini, 1990; Myhrer et al, 1992). However, a substantial period of time and intervening behavioural training and testing had elapsed, such that any beneficial effects would have diminished. Why this activity difference between the animals in the two experiements is so prevalent remains unclear.

Open field activity differences between enriched and isolated animals have been well documented. Isolated animals are generally more active, and their ambulation habituates more slowly within and across days than enriched animals (Dell & Rose, 1987; Einon et al, 1981; Einon et al, 1978). Although, some studies have found that the isolates initial open field activity is less active than other experimental groups (Dalrymple-Alford & Benton, 1981). Rearing behaviour has exhibited a more complex situation with some researchers finding isolates rearing more than enriched (Dalrymple-Alford & Benton, 1981; Dell & Rose, 1987), and at other times no significant difference between groups (Dell & Rose, 1987). The frequency of grooming behaviour is rearly examined.

The data from the open field observations in Experiment 2 shows behaviour frequencies much like what has been found in previous research, in that all animals habituated over days and within each day, but the Isolated animals to a lesser extent than the other differentially reared groups. The Isolated animals maintained a relatively high frequency of ambulation and rearing behaviour

throughout the 5 sessions, decreasing little over the days. As a result very little grooming behaviour was evident. The Enriched animals were much like the Isolated animals in that they maintained their ambulation and rearing frequency, but unlike the Isolated subjects, the Enriched animals also maintained a high frequency of grooming behaviour. The LTE animals decreased their ambulation quickly across and within days, the rearing behaviour followed suit, and grooming was also exhibited at a very low frequency.

What does appear to be problematic is the behaviour of the Enriched animals, in that the pattern of ambulation and rearing activity appears to resemble animals which have been isolated rather than enriched. At the start of open field testing the Enriched animals would have been in isolation for a period of two days which is not considered to be long enough for them to develop the characteristic hyperactivity normally associated with long-term isolation (Dalrymple-Alford & Benton, 1984b; Einon, 1980; Einon & Morgan, 1978b). But it must be considered that this period of isolation would have been the first encountered by these animals in their lifetime, and as such does appear to have had an effect on their behaviour.

CONCLUSION

In summary, the data generated in this research using a T-maze nonmatching-to-sample working memory task does not support the general notion of differing cognitive capacity among animals reared in different environments. However, only brief delay durations were examined, and therefore only the short-term aspects of working memory would have been affected. Longer delays of hours, or even days, may reveal differences between differentially reared subjects. Different tasks which examine the various aspects of declaritive memory may also find behavioural differences which may emerge in a task dependant manner. Only when these experimental contingencies have been examined will it be possible to determine the extent to which environmental rearing conditions contribute to differing cognitive capacities.

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